# ALUMINIUM INDUCED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN FRESHWATER FISH *CYPRINUS CARPIO* VAR. *COMMUNIS*

THESIS SUBMITTED TO
THE BHARATHIAR UNIVERSITY
FOR THE DEGREE OF
**DOCTOR OF PHILOSOPHY**

By
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# DECLARATION

I do hereby declare that the thesis entitled "**ALUMINIUM INDUCED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN FRESHWATER FISH, *CYPRINUS CARPIO* VAR. *COMMUNIS***" submitted to the Bharathiar University, Coimbatore, for the award of the Degree of **Doctor of Philosophy** in **Zoology**, is a record of original and independent research work done by me during the period of study from 1994-1999 under the supervision and guidance of **Dr.R.Manavalaramanujam**, and it has not previously formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any University.

Signature of the Candidate

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# CERTIFICATE

This is to certify that the thesis entitled "**ALUMINIUM INDUCED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN FRESHWATER FISH, *CYPRINUS CARPIO* VAR. *COMMUNIS***" is a record of original research work done by **Mrs. RANI, K.** in the Department of Zoology, as a full time research scholar during the period of study from 1994-1999 under my guidance and supervision for the award of the Degree of **Doctor of Philosophy** in **Zoology**. I further certify that this research work has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any University.

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# CONTENTS

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

Earth crust comprises 8% of aluminium, which is locked in minerals that conceal its status as the most abundant metal and the third most abundant element, after oxygen and silicon (Mason and Moore, 1982). In nature, it occurs only in combined form as an oxide in bauxite, the primary ore and in complex aluminosilicates such as micas and feldspars (Baes and Mesmer, 1976).

Aquatic pollution of freshwater by metals has become a serious problem because of harmful effects that high levels of such substances can have on aquatic organisms, including fishes (Hughes ***et al.,*** 1979). According to Martin (1994), until recently most natural waters contained insignificant amounts of aluminium, except for those in some volcanic regions and alum springs. Although aluminium is a common element, its concentrations in most fresh waters are usually very low (<1 mg/l) (Dougan and Wilson, 1974; Winge ***et al.***, 1977; Stumm and Morgan, 1981; Sadler and Lynam, 1986).

Acidification of catchment geology, often due to acidic deposition, leaches aluminium from its edaphic origin and concentrates in the aquatic environment of pore and surface waters (Cronan and Schofield, 1979). Acidification of soil water systems often causes an increased transfer of aluminium from edaphic to aquatic environments (Dickson, 1980; Seip ***et al.***, 1989; Verta ***et al.***, 1990), where Al may be present in different physico-chemical forms varying for instance in size

(Rosseland *et al.*, 1992; Poleo *et al.*, 1994) and charge properties (Lydersen *et al.*, 1987, 1994).

Mobilization of aluminium from the edaphic to the aquatic environment is an important consequence of acidic precipitation (Driscoll *et al.*, 1980). Such mobilization, however, is not the only route for anthropogenic introduction of aluminium to the hydrosphere (Heming and Blumhagen, 1988). Aluminium compounds in industrial processes, ash pond discharge, coal conversion effluents, water treatment works and dyeing of textiles may find their way into water courses, which exert severe toxicity to aquatic biota (Donaldson, 1974; Chu *et al.*, 1975; Hildebrand *et al.*, 1976; Birge, 1978; Boyd, 1979; Lamb and Bailey, 1981; Ganrot, 1986).

With the advent of acid rain, metal ions such as aluminium, mercury, and lead escape from mineral deposits and dissolve in fresh waters (Schofield and Trojnar, 1980; Haines, 1981; Hendershot *et al.*, 1986; McDonald *et al.*, 1989; Gagen *et al.*,1993). Acid rain serves as the key that springs the lock for metal ion release (Spry *et al.*, 1981; Christopherson *et al.*, 1982; Andersson and Nyberg, 1984; Sullivan *et al.*, 1986; Berqkvist, 1987; Sayer *et al.*, 1993; Gagen *et al.*, 1994; Peiffer *et al.*, 1997).

Hem and Roberson (1967) and Howells *et al.* (1990) reported that low concentration of aluminium in natural water is partly a consequence of the relationship between water pH and the solubility of aluminium minerals. Birchall *et al.* (1989) pointed that aluminium toxicity depends on the species of aluminium present

(cationic, neutral or anionic). Aluminium in solution forms various molecular species including the dominant species $Al^{3+}$, $AlOH^{2+}$, $Al(OH)_2^+$, and $Al(OH)_4^-$ depending largely on pH of water (Driscoll ***et al.,*** 1984; Seip ***et al.***, 1984; Lee, 1985; Driscoll and Schecher, 1988; Martin, 1991). According to Helliwell ***et al.*** (1983), Leivestad ***et al.*** (1987) and Sadler and Lynam (1987), the hydrolysis products, $Al(OH)^{2+}$ and $Al(OH)_2^+$ constitute the dominant parts of the inorganic dissolved aluminium which are most toxic at a pH level 5.0 to 6.0.

Howells ***et al.*** (1990) stated that in the pH range 5 to 8, aluminium has a very low solubility but outside this range solubility increases markedly. The possibility exists for two types of problems arising from aluminium in surface waters - (i) action of dissolved aluminium in acid or alkaline water outside the range pH 5.5 to 8 and (ii) the action of insoluble aluminium compounds in the form of suspended material between these pH values.

In waters of low pH (5.5) and low ionic strength, dissolved aluminium can reach concentrations which are toxic to aquatic organisms (Howells ***et al.***, 1983; Skogheim and Rosseland, 1984; Leivestad ***et al.***, 1987; Muniz ***et al.***, 1987; Carline ***et al.***, 1992a,b; Gagen ***et al.***, 1994). Such waters can arise from mine drainage, acid sulphate soil waters, geothermal waters, or poorly buffered lakes and streams receiving acid runoff (Schofield, 1977; Almer ***et al.***, 1978; Dickson, 1980; Grahn, 1980; Henriksen ***et al.***, 1988; Tipping and Hopwood, 1988).

Al-hydroxides constitute a substantial part of the dissolved aluminium between pH 5.0 and 6.0 when toxicity is most severe, and a rapid rise in pH substantially

increases the toxicity of aluminium to fish (Poleo *et al.*, 1994). Hence, under such conditions, it has been proposed that the process of ongoing Al polymerization is the major cause of acute Al toxicity to fish (Lydersen *et al.*, 1990b; Poleo and Muniz, 1993; Poleo *et al.*, 1991, 1994), and not as previously suggested, the monomeric Al-hydroxides themselves (Helliwell *et al.*, 1983; Leivestad *et al.*, 1987; Sadler and Lynam, 1987). Thus, the acute toxicity of aluminium to fish is a matter of reaction kinetics, where the degree of ongoing Al polymerization is the most important factor ( Lydersen *et al.*, 1991)

Similarly Al-OH polymers were identified as the prime cause of increased mortality of fish shortly after liming; liming is extensively used in the restoration of acidified lakes and streams to increase the water pH and alkalinity and also to reduce the concentration of toxic inorganic aluminium (Dickson, 1978, 1983). However, increased mortality of fish shortly after liming was observed in over saturated solution with ongoing polymerisation of low molecular weight aluminium species (McCahon *et al.*, 1989; Weatherly *et al.*, 1991).

Recently Flaten and Garruto (1992) and Parker and Bertsch (1992) have shown that the polynuclear aluminium species, tridecameric ion $[Al_{13}]^{7+}_{(aq)}$ is also responsible for acute aluminium toxicity in fishes. The observation was confirmed by Exley *et al.* (1994) that mononuclear $[Al_{13}]^{7+}_{(aq)}$ species is less toxic than other species.

Burrows (1977) and Ganrot (1986) while reviewing the role of aluminium in biology and chemistry explained that aluminium is present in very small

amounts in living organisms and in no case has it been shown to have a definite biological function; aluminium has generally been regarded as virtually biologically inert and the importance shown for its biochemistry, metabolism and toxicology has been limited. However, during recent years, an increasing number of toxic effects with aluminium have been established (Howells ***et al.***, 1983; Leivestad ***et al.***, 1987; Wood ***et al.***, 1988a, b and c; Rosseland ***et al.***, 1990; Farag ***et al.***, 1993; Atland, 1998).

Even though aluminium toxicity to fish was demonstrated more than 60 years ago, (Ebeling, 1928), the link between acidic Al-rich freshwater and fish mortality was established first by Schofield (1977). Similar results were obtained by subsequent studies by Dickson (1978), Dietrich and Schlatter (1989a), Vuorinen ***et al.*** (1994), Waring and Brown (1995) and Hyne and Wilson (1997).

The present understanding of the effect of aluminium toxicity in natural waters is based largely on a free ion activity model derived at chemical equilibrium (Pagenkopf, 1983; Schecher and Driscoll, 1987; Neville and Campbell, 1988). On the contrary, Exley ***et al.*** (1996) demonstrated that the strength of association of transient aluminium hydroxide polymers with gill mucous was the determining factor for acute toxicity rather than the free aluminium ions bound at the gill surface in non steady state characteristics of the natural environment (Exley and Birchall, 1992).

The primary target organs for toxicants are superficial sensory organs and the gills in fish (Skidmore and Tovell, 1972; Chevalier ***et al.***, 1985; Karlsson-Norrgren ***et al.***, 1986a, b; Galvez ***et al.***, 1998). Precipitated aluminium complexes can irritate the gill and cause inflammation, edema, swelling, and

sometimes irradiation of the secondary lamella, initiating a series of resistance mechanisms (Schofield and Trojnar, 1980; Jagoe *et al.*, 1987; Goossernaerts *et al.*, 1988). Potential sites of aluminium interaction at the lamellar epithelium can explain changing membrane fluidity and permeability characteristics, as well as intracellular uptake of aluminium and secondary effects on enzyme systems, intracellular calcium, and energy metabolism; the net result of the effects on these processes is in most cases is osmoregulatory failure (Muniz and Leivestad, 1980a,b; Rosseland and Skogheim, 1982; Fivelstad and Leivestad, 1984; Neville, 1985; Rosseland *et al.*, 1986a,b; Wood and McDonald, 1987; Wood,1989; Waring and Brown, 1995).

The toxic effects of aluminium in low pH on various parameters have been studied in brown trout, ***Salmo trutta*** (Dalziel *et al.*, 1986), in Atlantic salmon, ***Salmo salar*** (Birchall *et al.*, 1989; Berntssen *et al.* 1997), in rainbow trout, ***Oncorhynchus mykiss***, (Youson and Neville, 1987), in American brook trout, ***Salvelinus fontinalis***, (Wood *et al.*, 1988a, b and c; Baldigo and Murdoch, 1997), and in ***Coregonus wartmanni***, (Vuorinen *et al.*, 1990).

Aluminium is reported to be more toxic at alkaline range (pH >8 levels) in rainbow trout, ***Salmo gairdneri***, (Freeman and Everhart, 1977; Hunter *et al.*, 1980; Heming and Blumhagen,1988) and in zebra fish larvae, ***Brachydanio rerio***, (Dave, 1985). According to Hunn *et al.* (1987) at pH 7.5 aluminium significantly decreased the growth of brook trout, ***Salvelinus fontinalis***, after 45, and 60 days, and leading to a significant inhibition in spontaneous activity and feeding behaviour. Brook trout, ***Salvelinus fontinalis***, exposed to aluminium (200 µg/L Al) for 56 days accumulated

the metal within 3 days to its maximum concentration and eliminated aluminium from tissues more slowly at pH 7.2 than at pH 5.3 (Cleveland *et al.*, 1991a).

Freeman and Everhart (1977) reported that exposure of fish to aluminium (5.2 ppm) at pH 7.0 caused inhibition of feeding after 4 h of exposure. Activity was reduced after 4 days and hyperplasia of gills was observed after 7 days. Mortality occurred at a regular rate throughout the test period of 45 days and the survivors were extremely emaciated averaging only 24.1 per cent of the control weight as opposed to 91.8 per cent of the control.

Few studies to evaluate the toxicity of aluminium effluents were conducted on ***Daphnia magna*** (Biesinger and Christensen, 1972) on midge larvae, ***Tanytarsus dissimilis*** (Lamb and Bailey, 1981), ***Ceriodaphnia dubia***, ***Pimephales promelas*** (Hall and Hall, 1989) and rainbow trout, ***Salmo gairdneri***, largemouth bass, ***Micropterus salmoides*** and the marbled salamander, ***Ambystoma opacum*** (Birge *et al.*, 1980) at neutral and circumneutral pH. Some more references to be included

Several species of fish are susceptible to the adverse effects of aluminium as reflected in ~~the~~ blood composition changes, including ion loss (Neville and Campbell, 1988; Dietrich and Schlatter, 1989a; Vuorinen *et al.*, 1990; Waring and Brown, 1995),acid -base imbalance (Walker *et al.*, 1988; Wood *et al.*, 1988a, c; Playle *et al.*, 1989) hematological disturbance (Witters, 1986; Booth *et al.*, 1988) biochemical changes (Goss and Wood, 1988; McDonald *et al.*, 1991; Vuorinen *et al.*, 1992) and enzyme inhibition (Staurnes *et al.*, 1984; Reite and Staurnes, 1987; Rosseland *et al.*, 1992)

Call *et al.* (1984) and Hall and Hall (1989) remarked that aluminium toxicity studies conducted in dilute low pH water are not typical of most receiving systems. In waters with neutral pH when aluminium concentration exceeds the threshold limit of aquatic organisms, it becomes highly toxic with concomitant decrease in pH (Hunter *et al.*, 1980; Bielby, 1988; Allen and Sansom, 1989). Relatively less is known of the toxicity of aluminium under circumneutral condition (pH 6.5 - 7.5) at which neutral $Al(OH)_2$ and anionic $Al(OH)_4^-$ forms may predominate (Call *et al.*, 1984; Heming and Blumhagen, 1988).

From the foregoing review of literature it is understood that not much work has been done on aluminium induced physiological and biochemical changes in fish at neutral pH. Hence, the present work is designed to study the physiological and biochemical responses in fish, ***Cyprinus carpio*** var. ***communis***, exposed to aluminium toxicity in neutral soft water with reference to the following aspects:

1. 24 h median lethal concentration of aluminium sulphate to fish, ***Cyprinus carpio*** var. ***communis***.
2. Electrolyte disturbances and acid-base imbalance in the blood and plasma of fish during acute and sublethal exposure to aluminium as an indicator of iono-regulatory failure.
3. Changes in the hematological characteristics of the blood of fish treated with acute and sublethal concentration of aluminium sulphate to assess the fluid volume and oxygen uptake disturbances.

4. Biochemical and enzymatic alterations in the plasma and gills of fish, respectively, during acute and sublethal aluminium exposure to understand cellular and physiological dysfunction.

# BIOASSAY

# INTRODUCTION

Qualitative aspects of toxicology are important because they are fundamental to the safety evaluation process in which, one first determines the toxicologic profile of the substance and then establishes how the chemical can be employed safely to prevent injury (Plaa, 1982). According to Durham (1974), toxicity is the ability of a chemical molecule or compound to produce injury once it reaches susceptible site which is determined by the dosage. Cairns (1984) reported that from a regulatory point of view, toxicity tests are used for three major purposes; they are 1. screening of chemicals and products, ii. establishing limits and iii. monitoring; the author further stated that bioassay test can be used to establish the maximum acceptable concentration of a pollutant in a given environment without deliberate application of the chemical causing any unfavourable biological consequences.

Ramade (1987) in his review stated that many specialists have focussed their attention on the problem of bioassay in the monitoring of terrestrial and aquatic ecosystems. The aim of using bioassay in monitoring of environmental pollution is to establish a relationship between toxicity and concentration of a pollutant being studied in the biotope; the toxic effects can be divided into two categories *viz.*, effects that occur very quickly after a brief exposure to a chemical agent (acute) and those that appear only after repetitive exposure to the substances (chronic) (Durham, 1974; Ramade,1987; Nagel, 1993).

Plaa (1982) described that an acute effect is observed within the first several days of exposure to the agent; in most instances all these reactions are discernible within 1 to 2 weeks after administration; on the other hand chronic toxic properties of a substance may not be demonstrable until after several months of continuous repetitive exposures.

Brown (1980) stated that the ultimate response of an organism to acute exposure is dependent on the amount of toxicant at the critical target sites; the amount of toxicant at the target site is dependent in part, on the dose. The author added that between exposure to the dose and the response, there are four major occurrences *viz.*, absorption, distribution, metabolism and excretion; in this context, metabolism includes both toxification and detoxification processes.

Ramade (1987) explained the characteristic of acute toxicity as the cause of rapid death of contaminated individual or population by provoking the most drastic reaction, or very serious physiological disorder shortly after absorption through the skin or by the lungs or by mouth of a fairly large single dose or repeated dose of a poisonous compound.

Hagen (1959) pointed out the following objectives of acute toxicity tests:-

a. To assess toxicity in non - target species

b. To predict hazard to non-target species

c. To provide information on the mechanism of toxic action.

d. To provide data on which user risk benefit relationships may be assessed and

e. To ~~aid the~~ establish~~ment~~ ~~of~~ the exposure levels in studies designed to assess long term effects in experimental toxicology.

Toxicity can be calculated by determining the mortality rate after a fixed time as a function of increasing doses of the toxicant (Ramade, 1987). Different characteristic constants of the toxicant being studied can be determined by using dose mortality tests. The most important constant is the LC50 (median lethal concentration), which is the theoretical value, causing 50% mortality in the population being studied. The LC50 value is determined after 24 hours or 48 hours of exposure in tests of acute toxicity and in some cases after 96 hours (Ramade, 1987).

Exposure of animals to sublethal levels of pollutants may inflict stress on the mechanism required for maintaining a healthy physiological state; these changes may result in physiological, biochemical and behavioural processes, which will show a more accurate prediction of acceptable levels of pollutants in the environment (Koeman and Strik, 1975; Waterpaugh and Beitinger, 1985; Usha Rani and Ramamurthi, 1987; Mason , 1996).

Metals are mobilised from soil due to occurrence of acid rain (Haines, 1981; Spry ***et al.***, 1981). According to Cronan (1978) of the toxic metals, aluminium appears to be the primary element mobilised by strong acids of meteoric origin from the regions with acidic and base deficient soils.

Aluminium has been recognised as an extremely important toxic metal in many temperate freshwater environments but has received little attention in tropical aquatic ecosystem (Phillips, 1988). In recent years aluminium has come to be regarded as the major factor in the loss of fisheries in soft acid waters (Schofield and Trojnar, 1980; Driscoll, 1984; Oremerod ***et al.***, 1988; Dietrich and Schlatter, 1989a; Spry and Weiner, 1991; Cummins, 1994). Many Studies have revealed that aluminium toxicity depend more on pH and speciation (Sadler and Lynam, 1987; Neville and Campbell, 1988; Exley ***et al.***, 1994, 1996; Poleo, 1995). Exley ***et al.*** (1991, 1994), Exley and Birchall (1992) and Poleo (1995) have investigated the molecular mechanisms involved in the aluminium toxicity.

In the present study, the concentration - response relationship was studied by evaluating the median lethal concentration of aluminium to ~~fish~~, ***Cyprinus carpio*** var. ***communis***, where the response is mortality of fish.

# MATERIAL AND METHODS

Essential criteria for choosing test species for biotests should be stenoecious species because they are more vulnerable to any modification of the environment than are euryoecious species (Ramade, 1987). According to McDonald ***et al.*** (1989), the external surface of aquatic animals are structurally and physiologically delicate than ~~comparable to~~ liquid exposed surfaces in terrestrial animals. Aquatic animals have remarkable capacity of extracting and concentrating certain chemicals from water ***via***., respiration (Sharma, 1985). Hence, among aquatic test species, fish have been widely used as representative of toxicity studies for their ecological and economic importance, availability (Stebbing ***et al***., 1980), recreational value (EIFAC, 1969) and higher position in trophic level in the aquatic food chain (Forstner and Wittmann, 1983). Turing (1947) pointed out that fish are a very useful barometer of the real state of purity of water. By considering the above points, the right species should be chosen as a model, so that the observed results can be transferred to other species and the laboratory results can be extrapolated to field conditions (Nagel, 1993).

In order to extrapolate meaningful, relevant and ecologically significant results from aquatic toxicity tests not only appropriate tests but also appropriate organisms should be used; several criteria should be considered in selecting organisms for toxicity testing (Rand and Petrocelli, 1985).

1. Since sensitivities vary among species, species representing a broad range of sensitivities should be used whenever possible.

2. Widely available and abundant species should be considered.

3. Wherever possible, species should be studied which are indigenous to or representative of the ecosystem that may receive the impact.

4. Species that are recreationally, commercially and ecologically important should be included.

5. Species that are amenable to routine maintenance in the laboratory and techniques should be available for culturing and rearing them in the laboratory so that chronic toxicity tests can be conducted.

6. If there is adequate background information on a species (ie., its physiology, genetics and behaviour) the data from a test may be more easily interpreted.

**FISH MATERIAL**

Specimens of ~~The~~ scale carp, ***Cyprinus carpio*** var. ***communis***, (Linnaeus) ~~is~~ an exotic fish belonging to the family Cyprinidae were selected for the present investigation based on the following criteria.

1. The family Cyprinidae is well represented amongst the piscine inhabitants of the freshwaters of India.

2. It is ~~very~~ sensitive to environmental factors under consideration.

3. Readily available throughout the year in abundant quantity, commercially important and distributed throughout the world.

4. Breeds in confined waters.

5. Voraciously omnivorous, efficiently converts the food ingested into flesh, grows very fast, and prone to artificial feeds.

6. Tolerant to wide fluctuations of temperature and resistant to disease.

7. Hardy nature for handling and transport.

8. Suitable animal for bioassay testing.

**RECRUITMENT OF FISH FOR EXPERIMENT**

Healthy specimens of ***Cyprinus carpio*** var. ***communis***, were selected and transported to laboratory in polythene bags containing oxygenated water from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India. Fish of the same age and size from the same broodstock were collected.

Fish stock were maintained in a clean disinfected large rectangular tank previously cleaned with water and disinfected with potassium permanganate. They were acclimatized to laboratory conditions for a fortnight, before being used for experiments. Media were changed frequently to avoid fungal growth and contamination by metabolites. These stock were well aerated with aerators. During the acclimatization period, fish stocks were fed ***ad libitum*** with groundnut oil cake and rice bran powder in the ratio of 1:2. Feeding was stopped two days prior to

the commencement of experiments to keep the experimental animals more or less in the same state of metabolic requirement. During acclimatization, the fish stock was maintained at natural photoperiod and ambient temperature. From this stock fish with an average length of 6-7 cm and weighing 4-5 g were segregated and transferred to clean rectangular glass aquarium tanks (75 x 35 x 37 cm) of 150 L water capacity.

## TOXICANT

### Metal

According to Howells ***et al.*** (1990) aluminium is an important metal with the potential to replace zinc and tin in various end user industries; aluminium chloride is used as a pigment in the petroleum and organic chemical industries as a catalyst; aluminium sulphate is used in the pulp and paper industries and in treatment of potable water supplies where it acts as a coagulant.

Aluminium sulphate (anhydrous form) used for the present investigation was obtained from New India Chemical Enterprises, Kochi-682 024, India. The physical and chemical properties of aluminium sulphate (No.All429) are as follows:-

### Physical properties

a. Formula - $Al_2(SO_4)_3. 16H_2O$

b. Molecular weight - 342.15

c. Colour - White

d. Purity - 97% as $Al_2(SO_4)_3$

**Chemical properties**

According to Driscoll and Schecher (1988), aluminium in solution is amphoteric and can form both organic and inorganic complexes, tending to polymerize; it is a group III element found in solution only in the trivalent state; although it is metallic, it exhibits marked covalent tendencies and thus forming relatively stable complexes with a variety of inorganic and organic materials.

## WATER QUALITY

Tap water free from chlorine was used for the present study. The hydrobiological features such as temperature, pH, dissolved oxygen, total alkalinity, salinity and total hardness, were estimated for each set of experiment as these factors have a significant influence on the biodegradability and toxicity of pollutants.

## ANALYSIS OF WATER CHEMISTRY

Water temperature, pH, alkalinity, hardness, calcium and dissolved oxygen were monitored daily both in control and experimental tanks. Water temperature was measured by a thermometer. pH values were determined by a pen type pH meter (pH Scan 1,Eutech Cybernetics PTE Ltd, Singapore). Dissolved oxygen content was estimated by Winkler's method using starch indicator. Total alkalinity was determined using methyl orange as an indicator. Total hardness was estimated using Erichrome Black - T indicator and calcium level was determined using murexide indicator. Other parameters such as salinity, fluorine, silicate and dissolved organic carbon were estimated weekly. The above physico-chemical analysis of water used in the present experiments were carried out as per APHA ***et al.***

(1985). The analytical data for a single set of experiments for the above parameters are given in Table 1 as the value varied negligibly for the waters used for the other sets of experiments.

Table 1. Physico-chemical features of water used for the present investigation

| Physico-chemical features | Values |
|---|---|
| Temperature | 25.0±1.0 °C |
| pH | 7.5 ± 1 units |
| Dissolved oxygen | 7.1 ± 0.02 mg/L |
| Total alkalinity | 35.0 ± 5 mg/L |
| Salinity | 0.21 ± 0.1 ppt |
| Total hardness | 18.0 ± 0.5 mg/L |
| Calcium | 4.23 ± 0.5 mg/L |
| Fluoride | Nil |
| Silicate | 0.001 ppm |
| Dissolved organic carbon | Nil |

Values are means ~~of~~ ± S.E. of five individual observations

## BIOASSAY

Of the two types of bioassay experiments *viz.*, continuous flow (Mount and Warner, 1965) and static (APHA, 1971), the static bioassay method was chosen considering the limitations of the laboratory facilities.

### Toxicant preparation and exposure

Aluminium stock solutions were prepared by dissolving 1 gm of aluminium sulphate ($Al_2 (SO_4)_3.16 H_2O$) in 1 litre of tap water and stored in stoppered acid washed Erlenmeyer flasks. Tests were conducted in previously cleaned, sundried circular plastic tubs of 25 litres capacity. Preliminary toxicity tests were carried out to find out median lethal tolerance limit of fish to aluminium sulphate for 24 hrs. For determining the $LC_{50}$ concentration, plastic tubs holding 10 litres of water ~~was taken~~ were used,

and appropriate concentrations of aluminium sulphate were mixed to the test water in the tubs. In each tub 10 fish were introduced. A control tub was also maintained with 10 fish in 10 litres of water.

**Determination of median lethal concentration**

At the end of 24 h the survival/mortality of fish in the control and experimental tubs was recorded. The concentration at which 50% mortality of fish occurred after 24 h was taken as the median lethal concentration (LC50) for 24 h. The LC50 concentration for 24 h was calculated by the probit - analysis method of Finney (1978). Dead fish were removed immediately from the experimental tubs. Fish were ~~decided to be~~ considered dead when they lost their equilibrium, floating belly up, became immobile, cessation of ventilatory and mouth movements and inability to respond to any stimulus.

**Calculation of Regression line**

Critical concentrations or susceptibility of an organism to any toxicant can be estimated with sufficient accuracy from a probit/log concentration graph (Busvine, 1971). The two variables are plotted on a plain paper, or the original data (percentage mortality and concentration) can be plotted on a logarithmic probability paper. A straight line is fitted by eye and the critical concentrations are determined by inspection. Values determined ~~by~~ graphically are often remarkably close to calculated results, but they give no precise information on limits of accuracy. Based on the above method, the regression line was calculated in the present study.

# RESULT

According to Lloyd (1965) and Tabata (1969), ~~that~~ many physico-chemical parameters have a considerable influence on the toxicity and accumulation of metals in organisms. In general, toxicity of heavy metals in water is determined according to the toxicity of the metal itself, the synergistic and antagonistic aspects of the metal and the influence of physico-chemical parameters which determine a metal's availability by activation or deactivation.

Table 1 gives the data on the physico-chemical characteristics of water used for the present toxicity study. Table 2 and Fig. 1 present the data on probit regression / log concentration of fish, ***Cyprinus carpio*** var. ***communis***, when exposed to various concentrations of aluminium sulphate for 24 h and the same for 96 h is given in Table 3 and Fig. 2. The median lethal concentrations of $Al_2(SO_4)_3$ for 24 h was 44.90 ppm and for 96 h was 26.42 ppm. The chi-square test of Busvine (1971) on the toxicity data presented in Table 2 and 3 indicates clearly that fish population used for the experiment was homogenous.

Fish exposed to 24 h LC 50 concentration of aluminium sulphate showed a gradual retardation in their movement with extension of time. Profuse mucus production was observed. Towards the end of the experiments fish showed erratic movement, operculem was wide open, and lost equilibrium. Fish were apparently struggling hard for respiration and swam slantingly. Then jerking movement was observed before they died.

Table 2 : Calculation of log-concentration / probit regression line for 24 hours experiments in which fish ***Cyprinus carpio*** var. ***communis*** were exposed to different concentrations of aluminium sulphate

| Conc. of $Al_2(SO_4)_3$ (in ppm) | No. of fish used | % Mortality | Log conc. x | Empirical probit | Expected probit Y | Working probit y | Weight co-efficient | Weight W | Wx | Wy | $Wx^2$ | $Wy^2$ | Wxy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 10 | 28 | 1.6021 | 4.4120 | 4.4170 | 4.4200 | 0.5580 | 5.5800 | 8.9397 | 24.6636 | 14.3223 | 109.0131 | 39.5136 |
| 42 | 10 | 32 | 1.6232 | 4.5323 | 4.6600 | 4.5304 | 0.6010 | 6.0100 | 9.7554 | 27.2277 | 15.8350 | 123.3524 | 44.1960 |
| 44 | 10 | 46 | 1.6435 | 4.8996 | 4.9000 | 4.8992 | 0.6340 | 6.3400 | 10.4197 | 31.0609 | 17.1248 | 152.1737 | 51.0486 |
| 46 | 10 | 58 | 1.6628 | 5.2019 | 5.1320 | 5.2016 | 0.6340 | 6.3400 | 10.5422 | 32.9781 | 17.5296 | 1715391 | 54.8361 |
| 48 | 10 | 64 | 1.6812 | 5.3585 | 5.3580 | 5.3568 | 0.6160 | 6.1600 | 10.3562 | 32.9979 | 17.4108 | 176.7631 | 55.4760 |
| | | | | | | | | 30.4300 | 50.0132 | 148.9283 | 82.2224 | 732.8414 | 245.0703 |
| | | | | | | | | Sw | Swx | Swy | $Swx^2$ | $Swy^2$ | Swxy |

$\bar{x}$ = 1.6435
$\bar{y}$ = 4.8941
b = 11.878
Variance 'V' = 0.0002

$\chi^2$ = 0.331
Fiducial limits at 5% level
Lower limit $m_1$ = 42.13
Upper limit $m_2$ = 47.86
Regression equation 'y' = - 14.6266 + 11.8775 x

$LC_{50}$ 24 h value = 44.90 ppm
Values are means of five individual observations

Fig. 1. Log concentration of aluminium sulphate Vs per cent mortality of ***Cyprinus carpio*** var. ***communis*** and determination of LC 50 24 h value calculated from Table 2.

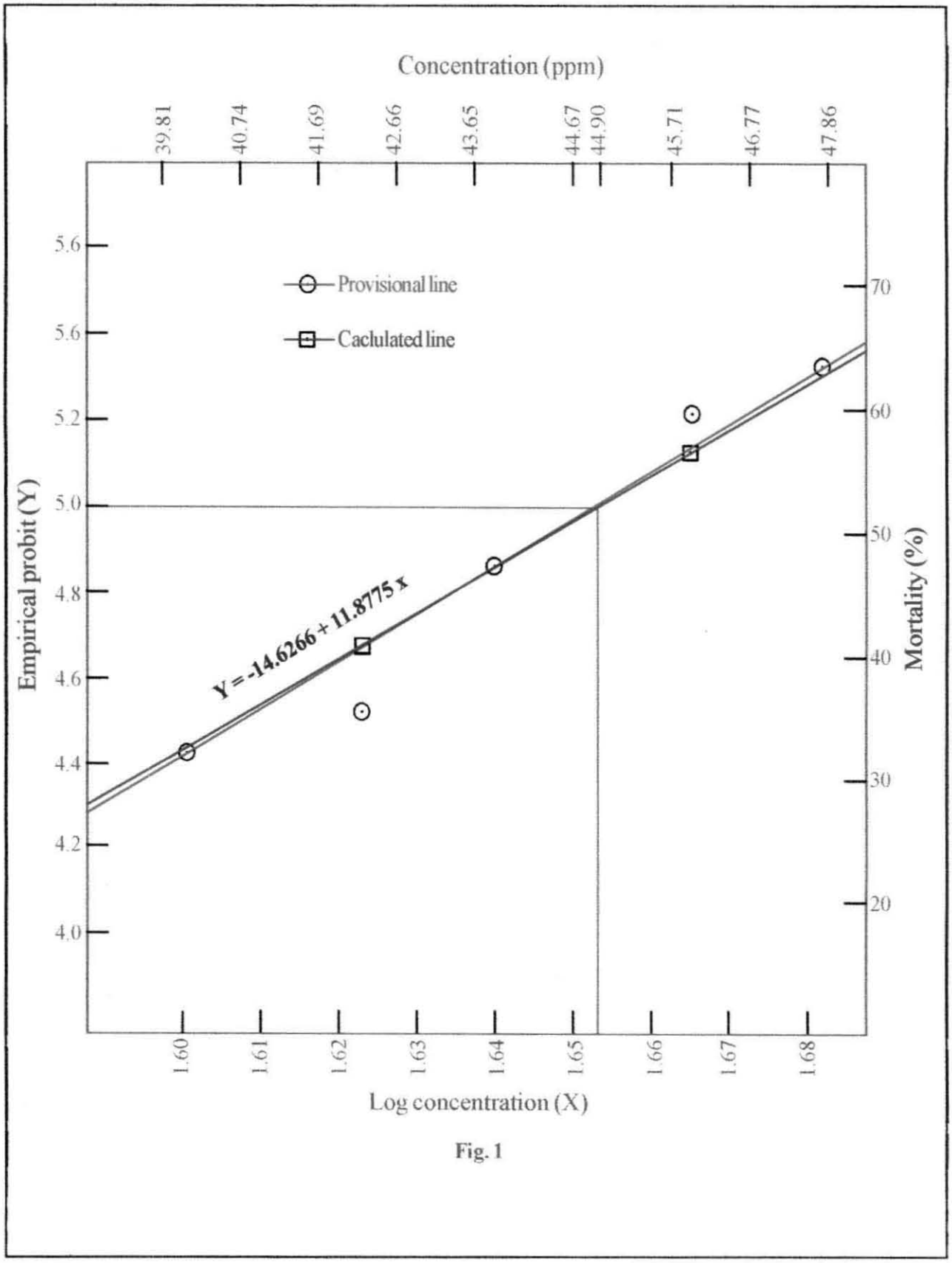

Concentration (ppm)
39.81
40.74
41.69
42.66
43.65
44.67
44.90
45.71
46.77
47.86
Provisional line
Caclulated line
Y = -14.6266 + 11.8775 x
Empirical probit (Y)
Mortality (%)
5.6
5.6
5.2
5.0
4.8
4.6
4.4
4.2
4.0
70
60
50
40
30
20
1.60
1.61
1.62
1.63
1.64
1.65
1.66
1.67
1.68
Log concentration (X)

Fig. 1

Table 3 : Calculation of log concentration / probit regression line for 96 hours experiments in which fish **Cyprinus carpio** var. **communis** were exposed to different concentrations of aluminium sulphate

| Conc. of $Al_2(SO_4)_3$ (in ppm) | No. of fish used | % Mortality | Log conc. x | Empirical probit | Expected probit Y | Working probit y | Weight coefficient | Weight W | Wx | Wy | $Wx^2$ | $Wy^2$ | Wxy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | 10 | 32 | 1342 | 4.53 | 4.53 | 4.528 | 0.581 | 5.81 | 7.7970 | 26.3076 | 10.4636 | 119.1211 | 36.3049 |
| 24 | 10 | 40 | 1.380 | 4.75 | 4.77 | 4.748 | 0.616 | 6.16 | 8.5008 | 29.2476 | 11.7311 | 138.8679 | 40.3617 |
| 26 | 10 | 48 | 1.414 | 4.95 | 4.95 | 4.949 | 0.634 | 6.34 | 8.9647 | 31.3766 | 12.6761 | 155.2830 | 44.3665 |
| 28 | 10 | 56 | 1.447 | 5.15 | 5.14 | 5.151 | 0.634 | 6.34 | 9.1739 | 32.6573 | 13.2747 | 168.2179 | 47.2551 |
| 30 | 10 | 64 | 1.477 | 5.36 | 5.33 | 5.356 | 0.616 | 6.16 | 9.0983 | 32.9929 | 13.4382 | 176.7102 | 48.7306 |
| | | | | | | | | 30.81 | 43.5347 | 152.5820 | 61.5837 | 758.2001 | 217.0188 |
| | | | | | | | | Sw | Swx | Swy | $Swx^2$ | $Swy^2$ | Swxy |

$\bar{x}$ = 1.4130
$\bar{y}$ = 4.9523
b = 6.0810
Variance 'V' = 0.0009

$\chi^2$ = 0.0118
Fiducial limits at 5% level
Lower limit $m_1$ = 23.1100
Upper limit $m_2$ = 30.3528
Regression equation 'y' = - 3.6401+ 60 810x

$LC_{50}$ 24 h value = 26.42ppm
Values are means of five individual observations

Fig. 2. Log concentration of aluminium sulphate Vs per cent mortality of ***Cyprinus carpio*** var. ***communis*** and determination of LC 50 96 h value calculated from Table 3.

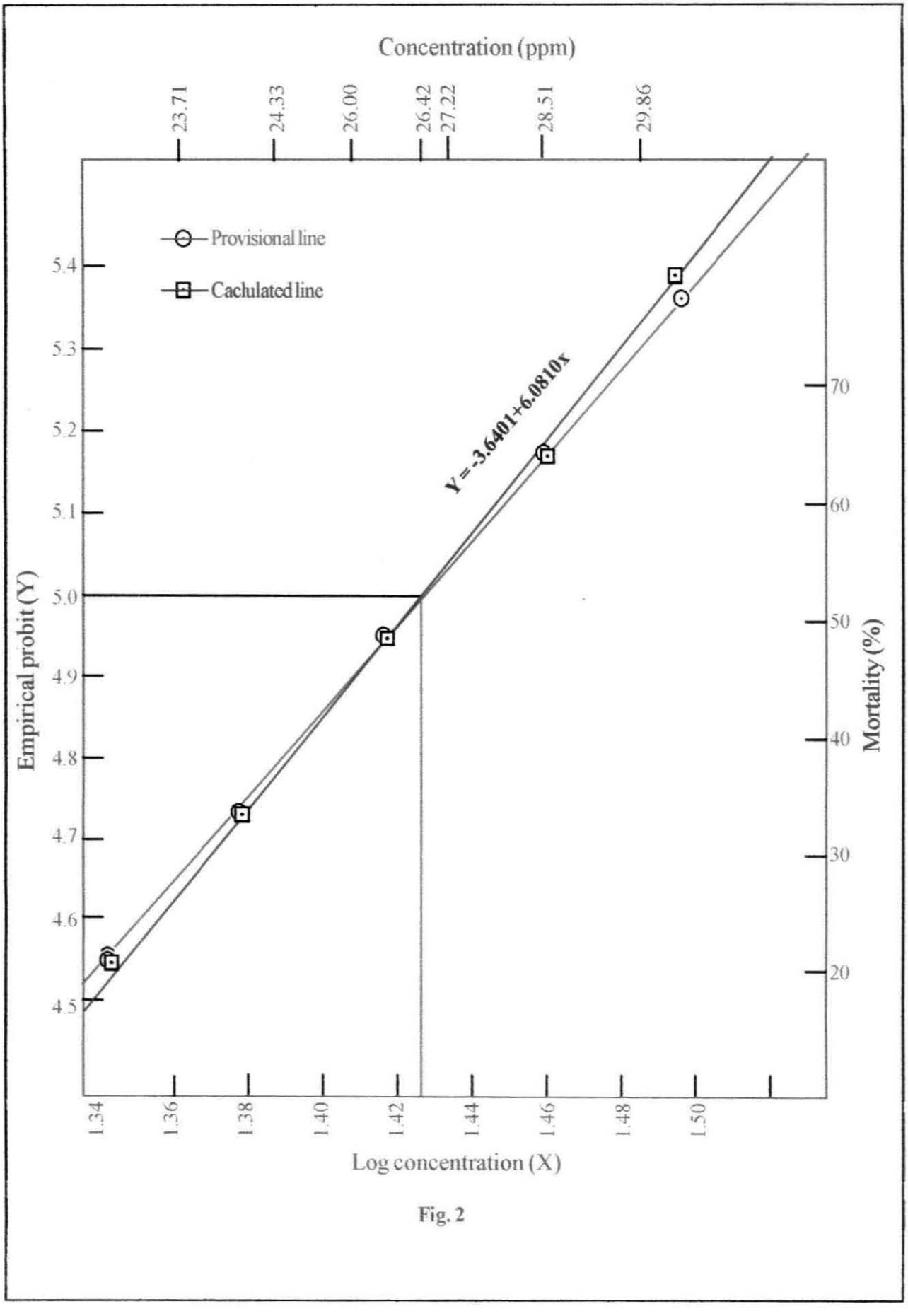

Concentration (ppm)
23.71
24.33
26.00
26.42
27.22
28.51
29.86
Provisional line
Caclulated line
Y = -3.6401+6.0810x
Empirical probit (Y)
Mortality (%)
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
70
60
50
40
30
20
1.34
1.36
1.38
1.40
1.42
1.44
1.46
1.48
1.50
Log concentration (X)

Fig. 2

# DISCUSSION

Bioassay can be defined as use of a living organism to measure the concentration of a substance in water by determining its potency in producing some specific effects in the organism (Alabaster and Lloyd, 1985).

Ferrando and Andreu (1991) reported that static acute toxicity tests provide rapid and reproducible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms which may form a data base for determining relative toxicity to a variety of species.

The median lethal concentration of aluminium sulphate to ***Cyprinus carpio*** var. ***communis***, for 24 h in the present investigation was determined as 44.90 ppm and for 96 h 26.42 ppm. Few authors have determined LC50 of aluminium by exposing fishes at neutral pH. Thomsen ***et al.*** (1988) calculated the LC 50 as 3.80 mg Al/l in softwater and 71.00 mg Al/l in hardwater in 25 day old larvae of ***Salmo gairdneri*** at pH 7.0. Similarly Birge ***et al.*** (1980) performed embryo larval bioassay on 11 trace metals of coal waste effluents in embryo of rainbow trout, ***Salmo gairdneri*** large mouth bass, ***Micropterus salmoides***, and the marbled salamander, ***Ambystoma opacum***, at pH 7.2-7.8. The LC 50 value of aluminium calculated for trout, bass and salamanders were 0.56, 0.17 and 2.28 ppm, respectively. Burrows (1977) has also given an account of lethal concentration of aluminium compounds to various species of freshwater fishes. The LC 50 value of aluminium chloride to rainbow trout, salmogairdneri, for 96h. was 7·4 and 14·6 PPM at pH 6·5 and 7·5, respectively (Call et al., 1984).

Most metals and their salts are simple inorganic compounds, the toxicity of which is caused by anions, cations or physicochemical properties of the salt; compounds of metals have an adverse effect on the self purification process of a water body; the harmful effect of salts of metals is manifested as forming precipitated insoluble hydroxides of metals deposited on the gills and causing mortality of fish, reducing the pH of water on hydrolysis and specifically imparting toxic effect based on specific action (Metelev *et al.*, 1983).

Aluminium toxicity depends on the species of aluminium present, which is largely dependent on water pH (Burrows, 1977) and the presence of complexing ligands such as fluoride (Driscoll *et al.*, 1980), silicic acid (Birchall *et al.*, 1989) and organic material such as humic acid (Gundersen *et al.*, 1994).

Two theories have been proposed by the previous workers to explain aluminium's toxic mode of action (Sadler and Lynam, 1987; Dietrich and Schlatter, 1989a; Handy and Eddy, 1989). The first one is physical effect, and the second one is surface binding. In the first theory inflammation of gill epithelial cells might have been caused by the precipitation of aluminium hydroxide at the gill surface. In the second one it was postulated that the hydrolysis products of Al *viz.*, $Al(OH)_2^+$, $Al(OH)^{2+}$ may bind to functional groups at the gill epithelium integral to membrane structure and function (Sadler and Lynam, 1987).

Several authors have observed similar symptoms of acute toxicity like inflammation, edema, swelling and in some cases lamellar fusion of the gills (Schofield and Trojnar, 1980; Karlsson-Norrgren *et al.*, 1986a; Jagoe *et al.*, 1987;

Goossernaerts *et al.*, 1988; Tietge *et al.*, 1988). In the present study also, after 24 h exposure significant structural alternations like edema and lamellar fusion of the gills were observed which might have led to mortality of fishes.

Profuse secretion of mucous by fish at low pH on aluminium exposures may clog the inter lamellar spaces in the gill epithelia, leading to reduced water flow over the respiratory surfaces and increased thickness of the diffusion barrier for gases (Westfall, 1945; Plonka and Neff, 1969; Daye and Garside 1976; Ultsch and Gros, 1979). Youson and Neville (1987) observed aluminium deposit on the gills impairing the gas exchange across the gills ~~resulting in failure of gas exchange~~ causing cellular anoxia, asphyxiation and acute death of fish. However, Exley *et al.* (1996) showed that aluminium precipitated at the gill surface was not always associated with an acute toxicity rather, the strength of association of aluminium with the gill epithelium occurring through the formation of hydroxy and oxo - bridges with oxygen based groups present in the mucous and gill is the determining factor.

$Al^{3+}$, a dominant aluminium ion at pH < 4.5 is implicated for acute aluminium toxicity in fish (Erichson and Jones, 1939; Neville, 1985; Dalziel *et al.*, 1987; Playle *et al.*, 1989). Exley *et al.* (1991) stated that the binding of $Al^{3+}$ at the gill epithelium is a pre-requisite for the acute toxicity.

It is reported that $Al^{3+}$ is less toxic than any other species of aluminium (Baker and Schofield, 1980; Muniz and Leivestad, 1980a, b; Schofield and Trojnar, 1980; Fivelstad and Leivestad, 1984; Wood and McDonald, 1987; NeVille and Campbell, 1988). However, Playle and Wood (1990) stated that $Al^{3+}$ bound to the gill

surface may undergo deprotonations to form aluminium hydroxide polymers due to changes in the pH of the gill microenvironment and cause toxicity to fish.

Several authors have reported that maximum toxicity occurs at around pH 5.0 to 5.5 where, aluminium hydroxide constitutes the dominant species (Schofield and Trojnar, 1980 ; Helliwell ***et al.***, 1983; Leivestad ***et al.***, 1987; Sadler and Lynam, 1987). Subsequently, aluminium polymers were identified as the major cause of acute aluminium toxicity in fish in the pH region 5.0 to 6.0 (Lydersen ***et al.***, 1990 a, b; Rosseland ***et al.***, 1992; Poleo and Muniz, 1993; Witters ***et al.***, 1996), contradicting earlier observations attributing toxicity to monomeric species of aluminium hydroxides.

Aluminium polymerization may start as soon as the $Al(OH)_2^+$ starts to yield high molecular species of aluminium (Hem and Robersen, 1967). Zaug (1982) and Playle and Wood (1989, 1990) stated that the gills may act as nucleating surfaces upon which aluminium can polymerize. Lydersen ***et al.*** (1991) found that the degree of ongoing polymerization is an important factor in determining acute toxicity of aluminium. Further, the authors reported that fish in contact with a freshly prepared solution of aluminium ~~is~~ are exposed to a spectrum of hydrated aluminium species, both monomeric and polymeric, ~~and~~ ~~these~~ solutions are highly toxic than aged solutions.

Recently, Exley ***et al.*** (1991) have described that phospholipids, a membrane transport protein, polyanionic mucous ~~consisting~~ containing negatively charged glycoproteins and sialic acid at the gill surface offer ~~as~~ potential binding sites for aluminium. The stability of binding is dependent on the relative concentrations of

competing cations and anions and intrinsic binding strength of aluminium to gill and mucous ligands where binding may be weak electrostatic or multiple co-ordination to a single aluminium ion (Birchall and Chappell, 1988).

In the present study, addition of LC 50 concentration of aluminium sulphate to the test water of pH 7.5±1, reduced the pH to 4.9±1. Similar drop in pH was observed by Hunter ***et al.*** (1980), Hall ***et al.*** (1985, 1987), Bielby (1988) and Hall and Hall (1989), while aluminium compounds were discharged into natural water. High concentration of aluminium in combination with low pH have been shown to cause mortality of freshwater fish in both field and laboratory studies (McCahon ***et al.***, 1989; Howells ***et al.***, 1990; Cummins, 1994; Atland and Barlaup, 1995). A similar drop in pH associated with elevated soluble aluminium concentration might have resulted in the mortality of fish of ***Cyprinus carpio*** var. ***communis*** in the present study.

Further, at this pH, the predominant species of aluminium present in the solution may be monomeric aluminium hydroxide, which might have started forming high molecular polymeric species. Growth of aluminium polymers on the gill surface and increased mucous secretion might have caused severe clogging of the interlamellar spaces leading to acute hypoxia and mortality of ***Cyprinus carpio*** var. ***communis*** in the present study supporting the observations of Zaug (1982), Playle and Wood (1989, 1990) and Lydersen ***et al.*** (1991) on the mechanism of acute toxicity in fish.

# ELECTROLYTE AND ACID-BASE BALANCE

# INTRODUCTION

The total amount of water retained in a body is maintained at constant level; the principal factors controlling the location and the amount of fluid in the various compartments are the osmotic forces which are maintained by the various solutes such as glucose, protein and electrolytes (Lehninger, 1982).

Fletcher (1970) and Haya and Waiwood (1983) stated that the electrolytes of the body fluids have various functions, such as regulating the osmotic pressure and acid-base balance of body fluids, preserving the permeability of cells, normal neuromuscular irritability and tissue functions.

$Na^+$ and $Cl^-$ are the major cation and anion of the extracellular fluid respectively, whereas $K^+$ is the major cation of the intracellular fluid. Sodium and potassium are considered as macroelements and they are essential to sustain biological life (Forstner and Wittman, 1983).

Circulating levels of sodium have been used as an indicator of stress, primarily due to the active movement of this ion across the gill structure depending on its concentration in the external medium (Bentley, 1971). Foskett ***et al.*** (1983) stated that blood $Cl^-$ is an important extracellular anion for the regulation of cation-anion balance and osmotic pressure. These inorganic electrolytes $Na^+$ and $Cl^-$ have been often used as an index of osmoregulation in freshwater teleost under conditions of stress (Umminger and Gist, 1973; Das and Das, 1976)

Hofer and Hoggett (1981) stated that high intracellular concentrations of $K^+$ regulates the body metabolism, the excitability of nerve and muscle cells, for the sustenance of osmotic balance and constancy of cell volume. According to Kay and Mommaerts (1960), Prosser and Brown (1961) and Freeman ***et al.*** (1968) variations in plasma potassium concentration would have widespread effects on the general body metabolism, altering the resting potential of most cells regulating their basal metabolism, glycolytic processes and regulating the production of mineralocorticoids.

Maintenance of constant internal ion concentration is important for active regulation of water influx and ion efflux in aquatic organisms (Mayer ***et al.***, 1992). Alterations in plasma electrolyte concentrations have proved to be a fairly reliable indicator of stress (McDonald ***et al.***, 1989; Mayer ***et al.,*** 1992, Folmar, 1993). By measuring changes in plasma ions levels, both acute and sublethal responses to pollutants can be effectively monitored; such measurements are easily made and the methods are inexpensive, fairly sensitive and provide good indices of pathological changes in ion regulating tissues (Larsson ***et al.***, 1985).

A bibliography on the effects of organic and inorganic chemical contaminants on electrolytes in teleost species has been provided by Folmar (1993). Plasma $Na^+$ level has been reported to rise ~~in the level~~ than control values after exposed to Hg, Cd and Pb in brook trout, ***Salvelinus fontinalis***, (Christensen ***et al.***, 1977) Cu in ***Heteropneustes fossilis*** (Singh and Reddy, 1990) and aluminium in ***Salvelinus fontinalis*** (Wood ***et al.***, 1988a). However, decreased plasma $Na^+$ level

were also recorded after exposure to hexavalent chromium in ***Salmo gairdneri*** (Van der Putte ***et al.***, 1982) and in some other fishes by various metals (Strik ***et al.*** 1975; Larsson ***et al.***, 1980)

Many studies have documented the progressive acidification of many lakes and the concomitant release of aluminium from the soil, which led to loss of fish population (Dietrich and Schlatter, 1989a; Sayer ***et al.***, 1993). In fish kills in natural acid waters, moribund fish have been found to have depressed plasma sodium and chloride levels (Leivestad and Muniz, 1976; Skogheim ***et al.***, 1984; Rosseland ***et al.***, 1994).

Aluminium exposure in the pH range of 4.3 to 6.0 depletes plasma and whole body $Na^+$ and $Cl^-$ content in rainbow trout ***Oncorhynchus mykiss*** (Dietrich and Schlatter, 1989a), in brook trout ***Salvelinus fontinalis*** (Cleveland ***et al.***, 1991b) in brown trout ***Salmo trutta*** (Sayer ***et al.***, 1991), in lake trout ***Salvelinus namaycush*** (Gunn and Noakes, 1987), in Atlantic salmon ***Salmo salar*** (Lacroix and Townsend, 1987; Lacroix ***et al.***, 1990), in cutthroat trout ***Oncorhynchus clarki*** (Woodward ***et al.***, 1991), in white fish ***Coregonus wartmanni*** (Vuorinen and Vuorinen, 1991; Faraq ***et al.***, 1993) and in perch ***Perca fluviatilis*** (Vuorinen ***et al.***, 1992). Aluminium has also been reported to decrease the plasma $Na^+$ and $Cl^-$ at alkaline pH 8.7 in ***Oncorhynchus mykiss*** as reported by Heming and Blumhagen (1988).

Potassium ion concentration in plasma and whole body declined from the normal value in acute and chronic exposure of fish to aluminium in acidic waters (Lacroix, 1985; Booth ***et al.***, 1988; Brown ***et al.***, 1990; Cleveland ***et al.***, 1991b, Sayer ***et al.***,

1991). Elevation of plasma potassium in rainbow trout, ***Oncorhynchus mykiss***, (Malte, 1986; Witters, 1986; Playle ***et al.***, 1989) and in brook trout, ***Salvelinus fontinalis***, (Wood ***et al.***, 1988a,b) was observed under aluminium exposure.

Heming and Blumhagen (1988) observed elevation of plasma potassium in rainbow trout, ***Oncorhynchus mykiss***, at pH 4.3+0.91 mg Al/l. Similarly moribund brown trout, ***Salmo trutta***, had higher plasma $K^+$ concentration than control after a 54 hr exposure to Al in acidic soft water (Reader ***et al.***, 1991). On the other hand, few reports have revealed that fish did not suffer ion loss despite exposing them to various levels of aluminium in acidic and alkaline pH (Malte, 1986; Heming and Blumhagen, 1988; Vuorinen and Vuorinen, 1991;Vuorinen ***et al.***, 1992; Waring and Brown, 1995).

Acid-base balance in teleost and elasmobranchs is a combination of $HCO_3^-$, $H^+$ and $NH_4^+$ transfer between the animal and environment, predominantly occurring at the gills to maintain a set point pH in the body fluids which is essential for maximum metabolic performance (Albers, 1970; Maetz, 1973; Cameron and Kormanik, 1982; Heisler, 1982; Wood ***et al.***, 1984). Any net production of acidic or alkaline metabolic products is balanced by equimolar elimination from the body fluids (Heisler, 1986).

pH is the central parameter of the acid-base status which is quite tightly regulated in different species at different $PCO_2$ values (Heisler, 1984, 1986). McDonald (1983a) pointed out that bicarbonate assumes a significant place because of its importance in $CO_2$ excretion and acid-base balance and its uptake at the gills is

equivalent to the excretion of $H^+$ which are controlled by the branchial mechanism of acid-base regulation.

The review of McDonald (1983a), Wood (1989) and Brown and Waring (1996) clearly depicts that there appears to be a relationship between blood acid-base balance and toxicant exposures.

Exposure of rainbow trout, ***Salmo gairdneri***, to acute concentrations of zinc produced a sharp decrease in blood pH, whereas with copper, a transient increase in pH was observed (Sellers ***et al.***, 1975). Playle ***et al.*** (1989) reported that exposure of rainbow trout, ***Salmo gairdneri***, to 105 mg $l^{-1}$ of aluminium caused a slight decrease in blood pH values with an apparent increase in $HCO_3^-$ level.

A severe decline in blood pH and bicarbonate levels was observed in brook trout ***Salvelinus fontinalis*** under acid+Al exposure by Walker ***et al.*** (1988) and Wood ***et al.*** (1988a, c) and in ***Salmo gairdneri*** by Heming and Blumhagen (1988). In contrast, even though acid and aluminium caused severe decreases in blood pH values in rainbow trout, ***Salmo gairdneri***, it exhibited increased external concentrations of $HCO_3^-$ (Heming and Blumhagen, 1988; Playle ***et al.***, 1989).

The foregoing review of literature indicates that much work has been done on the toxic effect of metals on ionic regulation and acid-base status in fish. Literature on the impact of aluminium toxicity in neutral pH on the above parameters are scanty. Hence, in the present work it is planned to study the influence of aluminium toxicity in neutral pH on the plasma electrolyte level and acid-base balance in ***Cyprinus carpio*** var. ***communis***.

# MATERIAL AND METHODS

## EXPERIMENTAL PROTOCOL

Two experimental series were performed *viz*., acute and sublethal toxicity studies. In each study one control and five replicates of treatment groups were maintained.

## ACUTE TOXICITY STUDIES

For acute toxicity studies, healthy fish were taken from the stock and were maintained in the glass tank. Then two days prior to experiments and during the experimental period feeding was discontinued. For acute aluminium toxicity studies, five circular plastic tubs with 25 L capacity were taken and each was filled with 20 L of water. The pH of the water was maintained at 7.5±1. Then LC 50 24 hr concentration of aluminium sulphate (44.90 ppm) was added to each tub and 20 fish were introduced in each tub and mixed well. For control groups, a circular plastic tub of 25 L capacity was taken with 20 L of water of normal pH 7.5±1. Then 20 healthy fish were selected from the stock and introduced into the tub and no toxicant was added.

## SAMPLING

At the end of 24 h, live fish from control and experimental tubs were taken and sacrificed for electrolyte studies. A minimum of 20 fish per treatment, 5 replicates per treatment and 20 fish per replicate were used for acute studies. Care was taken to avoid stress during sampling.

## SUBLETHAL TOXICITY STUDIES

A glass tank of 120 L capacity was taken and filled with 100 litres of water. The pH of the control and experimental work was maintained at 7.5±1. 1/10 of the LC 50 24 hr concentration of aluminium sulphate (4.49 ppm) was taken as the sublethal concentration (Sprague, 1971) and mixed with the water in the tank. One hundred Healthy fish ~~of 100 numbers~~ were selected from the stock and were introduced into the water tank. Five similar replicates were maintained. Water in the tank was changed daily and renewed with the toxic solution. A separate control was maintained by stocking 100 fish in a glass tank without adding toxicant. Fish were fed ***ad libitum*** every day. Water was changed daily.

## SAMPLING FREQUENCY

Experiments were conducted for a period of 35 days with a weekly sampling frequency. No mortality was observed during the experimental period. Upon completion of the stipulated exposure period of 7, 14, 21, 28 and 35 days, 20 fish were taken out and sacrificed without anesthetizing for further analysis. After removal of fish at various intervals of time, the volume of the experimental and control media were adjusted to maintain a constant density of fish per unit volume of water.

## COLLECTION OF BLOOD

Fish from experimental and control tubs were taken out for sublethal toxicity studies. Blood was drawn by cardiac puncture using plastic disposable syringe fitted with 26 gauge needle. The syringe and needle were prechilled and moist~~ured~~ened with heparin (Beparine$^{R}$ heparin sodium, IP 1000 IU $ml^{-1}$ derived from beef intestinal

mucosa containing 0.15% w/v chlorocresol IP preservative), an anticoagulant manufactured by Biological E Limited, Hyderabad, India. The collected blood was then decanted into ice cold heparinized plastic vials and placed immediately on ice. The blood samples from all the fish of their respective treatments were pooled for the analysis of different parameters. Blood samples were centrifuged at 10,000 rpm for 10 min. This left a clear yellow fluid, the plasma, which was used for the determination of protein, glucose, $Na^+$ $K^+$ ATPase, acid and alkaline phosphatases and chloride. Whole blood collected in vials were used for $Na^+$, $K^+$, pH, bicarbonate, hemoglobin, hematocrit, RBC and WBC studies.

## ESTIMATION OF ELECTROLYTES

Sodium, potassium and chloride ion content in the blood and plasma of fish, respectively, were analysed to study the electrolyte disturbance by acute and sublethal aluminium exposure in ***Cyprinus carpio*** var. ***communis***.

## ESTIMATION OF SODIUM

Sodium was estimated by flameless ion-selective electrode method using KNA2 Sodium-Potassium Analyzer (Radiometer Copenhagen, Denmark).

### Principle

The Radiometer's KNA2 Sodium-Potassium Analyzer is a computerized system for simultaneous determination of potassium and sodium using K and Na ion-selective electrodes (ISE). The ISE directly measures the concentration of potassium ($K^+$) and sodium ($Na^+$) in whole blood after manual dilution and also it

measures the activity of the ions in the aqueous phase by means of an F2211 Na SELECTRODE® (the displayed value is expressed as concentration of sodium ***i.e.***, in mmol/L). The Sealed Calomel Reference Electrode (K1702) is used as a reference electrode for both the K and the Na indicator electrodes.

For rinsing and zero point calibration of the K and Na electrodes, the S3641 rinse-and calibrating solution for normal $K^+/Na^+$, is used and S2422 calibrating solution is used for high $K^+$, low $Na^+$, for sensitivity calibration of the K and Na electrodes. The S4512 new salt-bridge solution is intended as a salt bridge between the reference electrode and the K and Na electrodes. The measuring unit with all the electrodes is thermostatted at 37 ± 0.1°C.

**Procedure**

The Sodium-Potassium Analyzer KNA2 was setup with mode button system to obtain P mode to estimate whole blood sodium. Then, the heparinized blood sample was aspirated through the inlet stub by using a catheter tube and immediately pressing the aspirate button, till the busy lamp glowed. Then the tube was removed and the inlet flap was turned off. The total sample needed for measurement is 125 µl. The sodium activity in the aqueous phase was measured by ISE (F2211 Na SELECTRODE®) within 47 sec. and the result was displayed as the concentration of sodium expressed in mmol/L.

## ESTIMATION OF POTASSIUM

Potassium was estimated using KNA2 Sodium-Potassium Analyzer (Radiometer Copenhagen, Denmark) by flameless ion-selective electrode method.

### Principle

The principle for potassium estimation is similar to that of sodium, but the activity of potassium in the aqueous phase is measured by means of an F2321 K SELECTRODE® (the displayed value is expressed as concentration ***i.e.***, mmol/L).

The S3641 rinse-and calibrating solution for normal $K^+/Na^+$ analysis is used for rinsing and zero point calibration of the K and Na electrodes. The S2422 calibrating solution for high $K^+$, low $Na^+$ is used for sensitivity calibration of the K and Na electrodes. The S4512 new salt-bridge solution is intended as a salt bridge between the reference electrode and the K and Na electrodes. The measuring unit with all the electrodes is thermostatted at 37 ± 0.1°C.

### Procedure

The mode button system was setup to obtain P mode to analyze whole blood potassium in the KNA2 sodium potassium analyzer. The sample (heparinized blood) was aspirated through the inlet stub using a catheter tube and pressing the aspirate button immediately till the busy lamp glowed. Then the tube was removed and the inlet flap was closed. The total sample needed for measurement is 125 µl. The potassium activity in the aqueous phase was measured by ISE (F2321

SELECTRODE®) within 47 sec. and the result was displayed as the concentration of potassium expressed in mmol/L.

## ESTIMATION OF CHLORIDE

Chloride was estimated by modified method of Schoenfeld and Lewellen (1964) and Levinson (1979) using Stangen (ECA 3) electrolyte diagnostic reagent kit manufactured and supplied by Stangen Immunodiagnostics, Hyderabad, India (Code : 12175) using Photometer 4010 System (Boehringer Mannheim GmbH, West Germany).

### Principle

Chloride reacts with mercuric thiocyanate and ferric nitrate to form undissociated mercuric chloride and allow the formation of a red brown ferric thiocyanate complex. The intensity of the brown color produced is proportional to the chloride concentration in the sample.

The reaction can be represented as follows :

$$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2SCN^-$$

$$3SCN^- + Fe^{3+} \longrightarrow Fe(SCN)_3 \text{ (red brown)}$$

### Reagents

Reagent 1 : Chloride reagent

Reagent 2 : Standard chloride reagent (100 mmol/L)

**Procedure**

Four test tubes labelled blank, standard, control and test were taken and to each tube 2000 µl of chloride reagent (Reagent 1) was added. Then, 10 µl of deionised water was added to blank and to standard, 10 µl of standard chloride reagent (Reagent 2) was added. Similarly 10 µl of plasma from control and experimental groups was added to control and test tubes, respectively. The contents in the tubes were mixed well and allowed to stand at room temperature for 2 min. and the absorbance of standard, control and test was measured at 505 (Hg 546 nm) against blank using Photometer 4010 System.

**Calculation**

$$\text{Chloride in mmol/L} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

Concentration of standard chloride = 100 mmol/L

**ESTIMATION OF BLOOD pH**

Blood pH was estimated by using ABL300 Acid-Base Laboratory (Radiometer Copenhagen, Denmark).

**Principle**

The ABL300 Acid-Base Laboratory is an automated and computerized micro blood-gas and acid-base balance analyzer which measures pH, $PCO_2$, $PO_2$ by their respective electrodes and Hb by hemoglobin photometer on blood samples and

calculates $HCO_3^-$, $TCO_2$, ABE (Actual Base Excess), SBE (Standard Base Excess), SBC (Standard Bicarbonate), SAT (Oxygen Saturation), $O_2CT$ (Oxygen Content) from the measured values.

$$pH = pH(m) + 0.012$$

The measured pH is calculated as follows:

$$EpH(m) - E'pHR$$

$$pH(m) = S'' \times 0.615 + pH'R^0$$

Where,

| | | |
|---|---|---|
| pH(m) | = | Measured pH in the sample |
| EpH(m) | = | Electrode chain potential (mV) in the sample |
| E'pHR | = | Electrode chain potential (mV) in the red calibrating solution (from the last 1- or 2-point calibration) |
| S'' | = | Electrode sensitivity in % (from the last 2-point calibration) |
| $pH'R^0$ | = | Calculated red pH value from the last 1- or 2-point calibration |

The pH of the blood is measured by G2701 pH electrode. The K1701 Calomel Reference Electrode is used as the reference electrode for the G2701 pH electrode.

The S1546 green calibrator solution (for low pH) and S1556 red calibrator solution (for high pH) were used as the calibrator solutions. The (KCl) S4112 salt-bridge solution used as a salt-bridge between the reference cell and the sample with which the glass electrode is in contact. The S4706 rinse solution was used

for rinsing. All these solutions were provided by Radiometer Copenhagen, Denmark. All measurements and calibrations were carried out at 37 ± 0.1°C.

**Procedure**

The ABL300 Acid-Base Laboratory was used to obtain the ready lamp (green) to glow to analyze whole blood pH value. Then, the sample, (heparinized blood) was aspirated using a catheter tube through the inlet stub and immediately pressing the aspirate button till the sample lamp (yellow) glowed. The tube was then removed and the inlet flap was closed. The total sample required for measurement was 85 µl. The pH was measured by G2701 pH electrode within 171 sec. and the result was displayed as the actual pH according to pH scale or in nmol/L.

**ESTIMATION OF BLOOD $HCO_3^-$ (BICARBONATE)**

Blood $HCO_3^-$ (bicarbonate) was analysed using ABL300 Acid-Base Laboratory (Radiometer Copenhagen, Denmark).

**Principle**

The ABL300 Acid-Base Laboratory is an automated and computerized micro blood-gas and acid-base balance analyzer which measures pH, $PCO_2$, $PO_2$ by their respective electrodes and Hb by hemoglobin photometer on blood samples and calculates $HCO_3^-$, $TCO_2$, ABE (Actual Base Excess), SBE (Standard Base Excess), SBC (Standard Bicarbonate), SAT (Oxygen Saturation), $O_2CT$ (Oxygen Content) from the measured values.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The actual concentration of the bicarbonate can be calculated from the Henderson-Hasselbalch equation if the pH and $PCO_2$ are known (Siggaard-Andersen, 1974).

$$pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

In order to establish a linear relationship between pK and pH, the above formula from Siggaard-Andersen (1974) is used.

The ABL300 then uses this formula to calculate $HCO_3^-$:

$$\left(\frac{pH - 6.161}{0.9524}\right)$$

$$[HCO_3^-] = 0.0306 \times PCO_2 \times 10$$

Actual bicarbonate includes, bicarbonate, carbonate and carbamate in plasma and is expressed in mmol/L (=meq/L).

The S1546 green calibrator solution (for low pH) and S1556 red calibrator solution (for high pH) are used as the calibrator solutions. The S4112 (KCl) salt-bridge solution serves as a salt-bridge between the reference cell and the sample with which the glass electrode is in contact and S4706 rinse solution serves in rinsing. All these solutions are provided by Radiometer Copenhagen, Denmark.

**Procedure**

The ABL300 Acid-Base Laboratory was setup to obtain the ready lamp (green) to glow to analyze whole blood bicarbonate value. Then, heparinized blood sample was aspirated through the inlet stub using a catheter tube and immediately pressing the aspirate button. The sample was aspirated till the sample lamp (yellow) glowed. Then the catheter tube was removed and the inlet flap was closed. The total sample needed for measuring all the parameters is only 85 µl. The actual bicarbonate ($HCO_3^-$) concentration was calculated using the measured values of $PCO_2$ and pH of the blood sample and the result was displayed within 171 sec. as the actual bicarbonate concentration expressed in mmol/L.

**STATISTICAL ANALYSIS**

~~Each~~ Data were analysed statistically and the mean value of five individual observations was taken for each parameter. The significance of sample mean between control and treated fish was tested using students 't' test (Campbell, 1981). The standard error for the sample mean was calculated and they are given in the appropriate tables. The significance of sample mean between control and the treatment were analysed by analysis of variance (two way anova for sublethal studies) using Stata Mnu Menu of Computer Resource Centre, Santa Monica, California, USA (S1No.22103753, Yr. 1984-1990) followed by Duncan's multiple range test (DMRT) of Duncan (1951). Similar statistical analysis was applied to all the data for Chapter IV, V and VI. The analytical data together with tables and graphs / bar-diagrams for all the chapters are presented in the appropriate places in the text.

# RESULT

Changes in electrolyte and acid-base balance in the blood and plasma of fish, ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium stress ~~is~~ are presented in Table 4 and Fig. 3. Blood sodium and plasma chloride level in the experimental fish showed 12.64% and 57.01% decrease ~~than~~ from that of their control groups, respectively. Plasma chloride in experimental group showed nearly five fold decrease than that of sodium. However, Potassium level increased by 10.82 % than that of the control. Similarly both pH and bicarbonate levels of blood of fish declined in the experimental group ~~than that of~~ compared to their respective controls giving a value of 0.93% and 46.15 %, respectively (Table 5 and Fig. 4). All the values were found to be significant at 5% level.

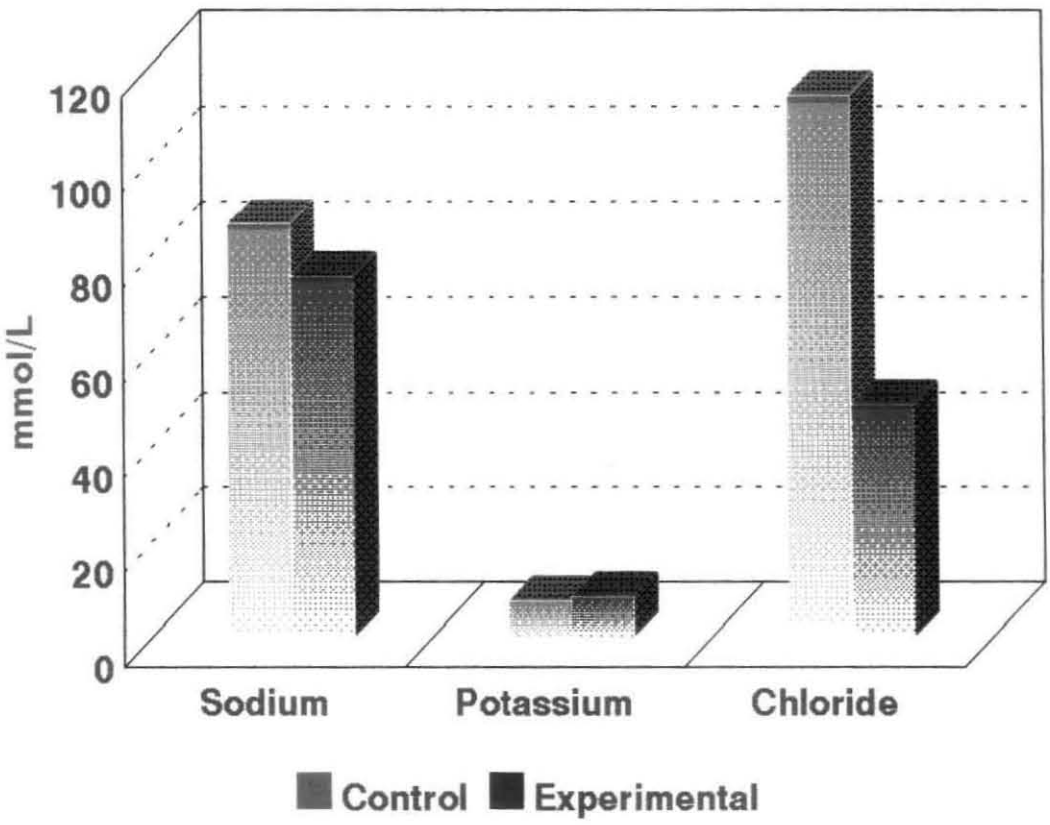
Sublethal treatment of fish with aluminium sulphate caused an initial decline in blood sodium level ~~than that of~~ from its control giving a reduction of 22.94% at the end of 7th day (Table 6 and Fig. 5). However, after 7th day, blood sodium level increased gradually showing 22.57% in the tested group over that of its control at the end of 35th day. Analysis of the data by Duncan's Multiple Range Test (DMRT) revealed that sodium level varied significantly from control during all the exposure periods (Table 7). At the end of 14th day, it was on par with that of control. When both the treatments were analysed for varying periods, the following results were observed: in control groups, $Na^+$ levels varied significantly on 14th and 35th day, 35th and 7th day, 28th day and 21st day whereas, 35th and 28th day, 28th and 7th day and 7th and 21st day were on par with each other. When treatments were analysed, 7th

Table 4 : Sodium, potassium and chloride levels in the blood and plasma of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.

| S. No. | parameters | Control | Experimental | Per cent change | Calculated 't' |
|---|---|---|---|---|---|
| 1 . | Sodium (mmol/L) | 87.000 ± 1.581 | 76.000 ± 1.140 | -12.64 | 5.6429* |
| 2 . | Potassium(mmol/L) | 7. 850 ± 0.074 | 8.700 ± 0.352 | +10.82 | 2.3627* |
| 3 . | Chloride(mmol/L) | 114.000 ±1.304 | 49.000 ± 1.892 | - 57.01 | 28.2688* |

Values are mean ± S. E. of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control
* Values are significant at 5% level
Degrees of freedom at 8 t 0.05 = 2.306.

Fig. 3. Sodium, potassium and chloride levels in the blood and plasma of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.



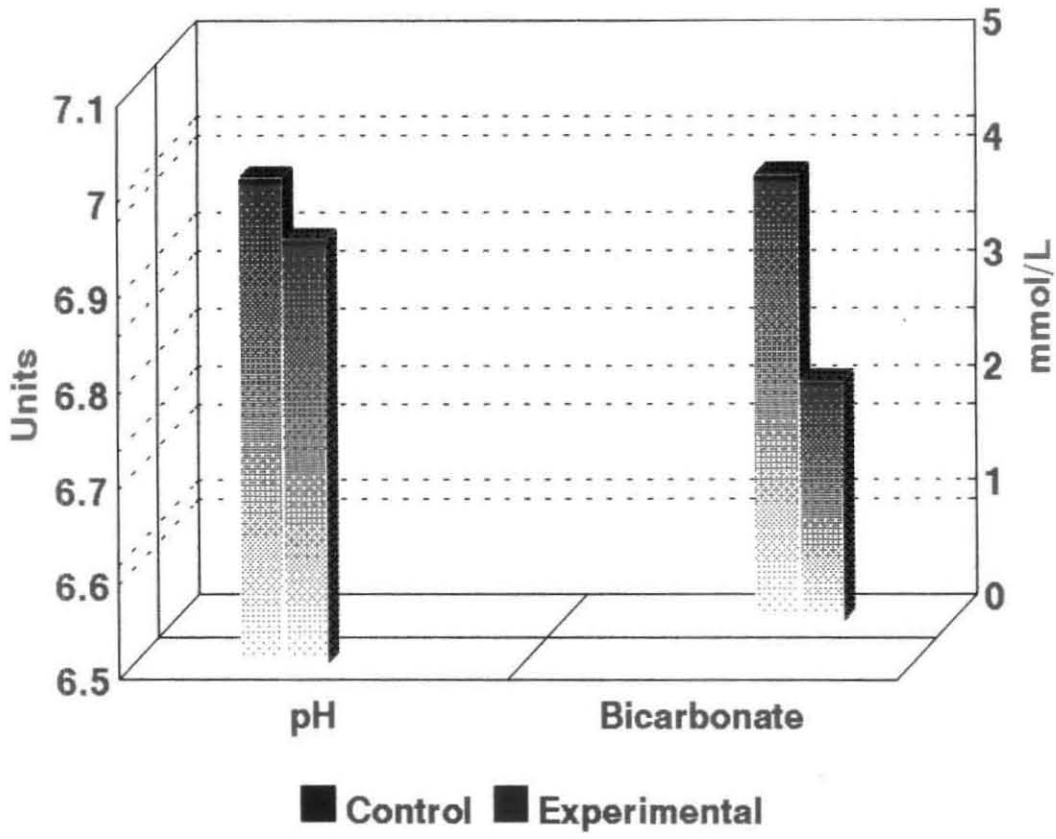

Sodium, potassium and chloride levels in the blood and plasma of *Cyprinus carpio* var. *communis* exposed to acute aluminium sulphate toxicity

Table 5 : pH and bicarbonate levels in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.

| S. No. | Parameters | Control | Experimental | Per cent change | Calculated 't' |
|---|---|---|---|---|---|
| 1. | pH (units) | 7.010 ± 0. 005 | 6. 945 ± 0.017 | -0. 93 | 3. 7696* |
| 2. | Bicarbonate (mmol/L) | 3.900 ± 0.212 | 2.100 ± 0.158 | -46.15 | 6. 8034* |

Values are mean ± S. E. of five individual observations
- Denotes per cent decrease over control
* Values are significant at 5% level
Degrees of freedom at 8 t 0.05 = 2.306.

Fig. 4. pH and bicarbonate levels in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.




pH and bicarbonate levels in the blood of *Cyprinus carpio* var. *communis* exposed to acute aluminium sulphate toxicity

Table 6 : Sodium content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

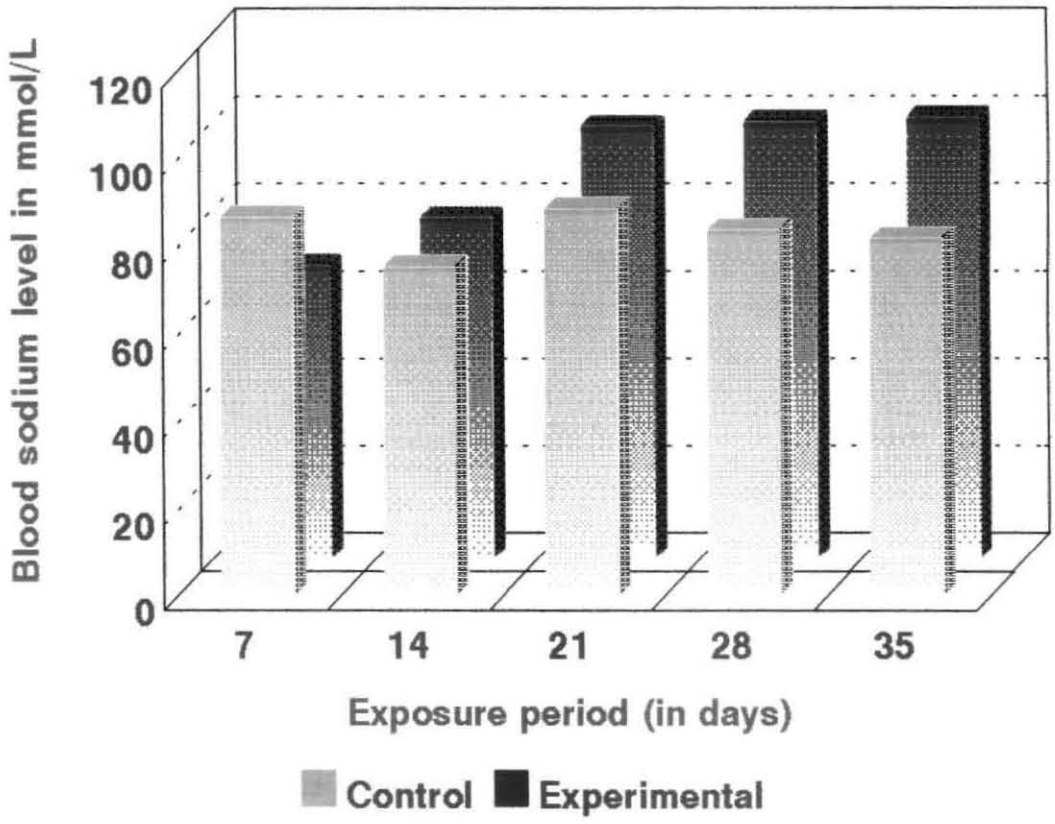
| Exposure period (in days) | Blood sodium level in mmol/L | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 87.200±1.854 | 67.200±1.049 | -22.94 |
| 14 | 76.000±1.225 | 78.000±1.581 | +2.63 |
| 21 | 89.000±1.643 | 99.000±0.894 | +11.24 |
| 28 | 84.200±0.735 | 100.000±2.025 | +18.76 |
| 35 | 82.400±2.135 | 101.000±1.140 | +22.57 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 5. Sodium content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Sodium content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 7 : DMRT table for sodium level in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $87.200^{CDa}$ | $67.200^{Ab}$ | $77.200^{I}$ |
| 14 | $76.000^{Aa}$ | $78.000^{Ba}$ | $77.000^{I}$ |
| 21 | $89.000^{Da}$ | $99.000^{Cb}$ | $94.000^{II}$ |
| 28 | $84.200^{BCa}$ | $100.000^{Cb}$ | $92.100^{II}$ |
| 35 | $82.400^{Ba}$ | $101.000^{Cb}$ | $91.700^{II}$ |
| Mean | $83.760^{\alpha}$ | $89.040^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at F <0.05
CV = 3.906 %

and 14th days varied significantly from each other and also from that of 21st, 28th and 35th day, but the exposure periods were on par with other.

When the period means were analysed irrespective of the treatments 7th, 14th and 21st, 28th and 35th day were on par with each other and varied significantly from each other. When treatment means were analysed, the $Na^+$ level varied significantly than that of the control. The analysis clearly depicts that $Na^+$ level varied significantly than that of the control.

Table 8 and Fig. 6 give the data on potassium levels from the blood of fish exposed to the sublethal concentration of aluminium sulphate. The $K^+$ level increased from 14.10% at the end of 7th day to 30.39% at the end of 35th day. Analysis of changes in $K^+$ level by DMRT depicts that it varied significantly in aluminium exposed fish than that of the control in all the exposure periods except on 7th day (Table 9).

When control alone was analysed, 7th and 21st days, 21st and 14th days, 14th and 35th days and 28th and 35th days were on par with each other. The rest of the combinations varied significantly among themselves. When treatment groups alone were analysed 14th and 28th day, 28th and 35th day were on par with each other. The rest of the combinations varied significantly from these groups and among themselves.

When period means were analysed, irrespective of their treatments, all the exposure periods varied from each other except 28th and 35th day which were on par with each other. When treatment means were analysed blood $K^+$ level varied

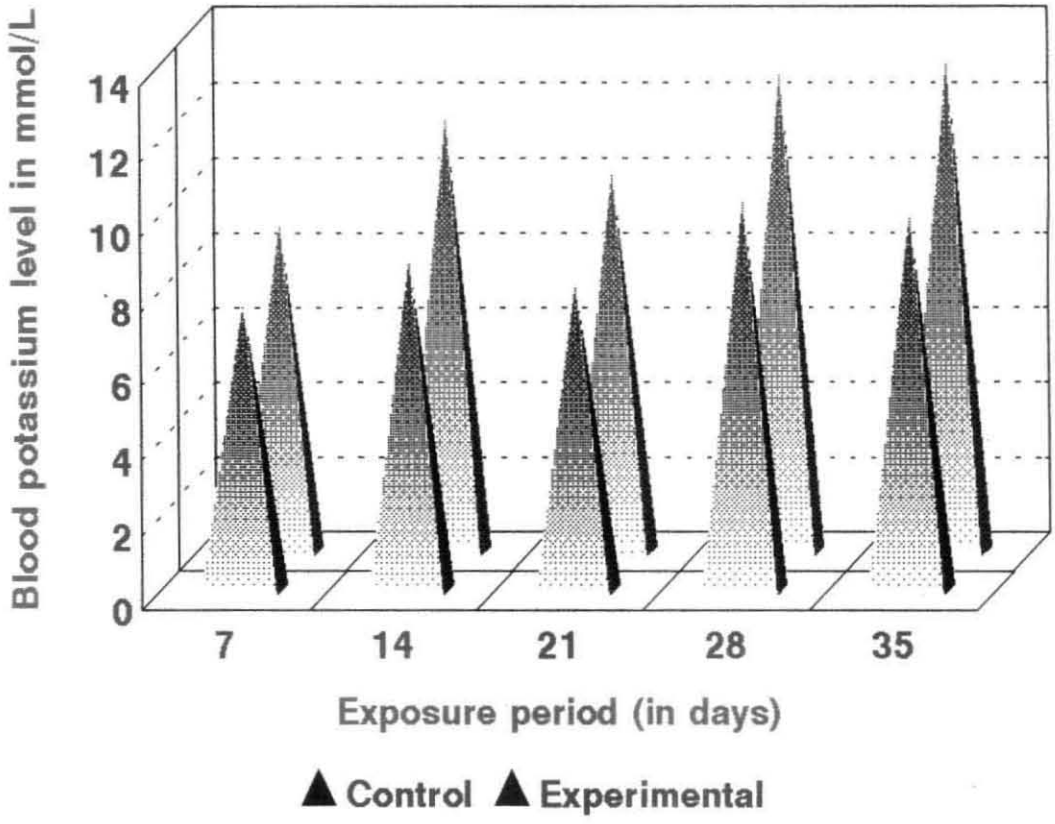
Table 8 : Potassium level in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Blood potassium level in mmol/L | | Per cent change |
|---|---|---|---|
| | Control | Experimental | |
| 7 | 7.800±0.381 | 8.900±0.245 | +14.10 |
| 14 | 9.000±0.161 | 11.800±0.228 | +31.11 |
| 21 | 8.300±0.200 | 10.300±0.376 | +24.10 |
| 28 | 10.600±0.502 | 13.000±1.025 | +22.64 |
| 35 | 10.200±0.596 | 13.300±0.164 | +30.39 |

Values are mean ± S . E . of five individual observations

'+' Denotes per cent increase over control

Fig. 6. Potassium content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Potassium content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 9 : DMRT table for potassium level in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate at varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $7.800^{Aa}$ | $8.900^{Aa}$ | $8.350^{I}$ |
| 14 | $9.000^{ABa}$ | $11.800^{Cb}$ | $10.400^{III}$ |
| 21 | $8.300^{Aa}$ | $10.300^{Bb}$ | $9.300^{II}$ |
| 28 | $10.600^{Ca}$ | $13.000^{CDb}$ | $11.800^{IV}$ |
| 35 | $10.200^{BCa}$ | $13.300^{Db}$ | $11.750^{IV}$ |
| Mean | $9.180^{\alpha}$ | $11.400^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at F <0.05
CV = 10.042 %

significantly than that of the control thus indicating that $K^+$ level increased during sublethal treatment with aluminium.

Chloride content in the plasma of all treated fish declined drastically by 55.82% from control values after 7th day (Table 10 and Fig. 7). However, it recovered slightly giving a per cent decline of 44.03% from control values at the end of the 35th day

DMRT analysis of chloride level shows that plasma chloride level varied significantly during all the exposure periods (Table 11). Analysis of control alone for varying exposure period revealed that $Cl^-$ level varied during all the exposure period except 14th and 21st days, which were on par with each other. Similarly analysis of sublethal aluminium treatment alone for varying experimental periods showed that decrease in $Cl^-$ level was significant only at the end of 7th and 14th day, which were also significantly different from that of 21st, 28th and 35th days. On the other hand, the latter exposure periods were on par with each other.

Analysis of period means irrespective of treatments showed that the 7th and 14th day were on par with each other but varied from that of the rest of the exposure periods, which also varied among themselves. Similarly analysis of treatment means showed that sublethal concentration of aluminium sulphate caused a change in $Cl^-$ level which varied significantly than that of the control, indicating the aluminium sulphate is highly toxic by depressing $Cl^-$ level in fish at sublethal stress.

Blood pH level of ***Cyprinus carpio*** var. ***communis*** increased upto the end of 21st day during sublethal aluminium treatment (+2.48%) with relative to the ~~than~~ control (Table 12 and Fig.

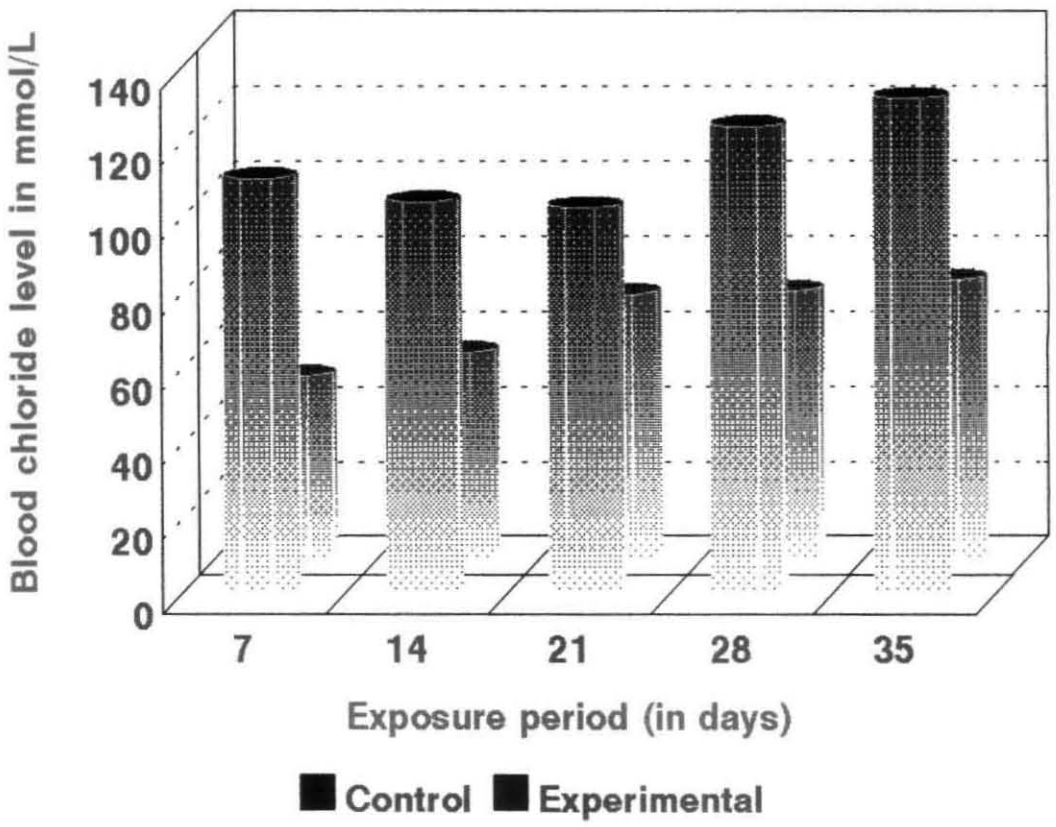
Table 10 : Chloride level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Plasma chloride level in mmol/L | | |
|---|---|---|---|
| | Control | Experimental | Per cent change |
| 7 | 112.600±0.758 | 49.750±0.575 | -55.82 |
| 14 | 106.400±1.610 | 56.000±1.844 | -47.57 |
| 21 | 104.800±1.751 | 71.310±0.456 | -31.82 |
| 28 | 126.400±1.071 | 72.180±0.294 | -42.90 |
| 35 | 134.000±1.491 | 75.000±1.673 | -44.03 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

Fig. 7. Chloride content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Chloride content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 11 : DMRT table for chloride level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $112.600^{Ba}$ | $49.750^{Ab}$ | $81.175^{I}$ |
| 14 | $106.400^{Aa}$ | $56.000^{Bb}$ | $81.200^{I}$ |
| 21 | $104.800^{Aa}$ | $71.310^{Cb}$ | $88.050^{II}$ |
| 28 | $126.400^{Ca}$ | $72.180^{Cb}$ | $99.290^{III}$ |
| 35 | $134.000^{Da}$ | $75.000^{Cb}$ | $104.500^{IV}$ |
| Mean | $116.840^{\alpha}$ | $64.848^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at F <0.05
CV = 3.152 %

Table 12 : Blood pH of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate.

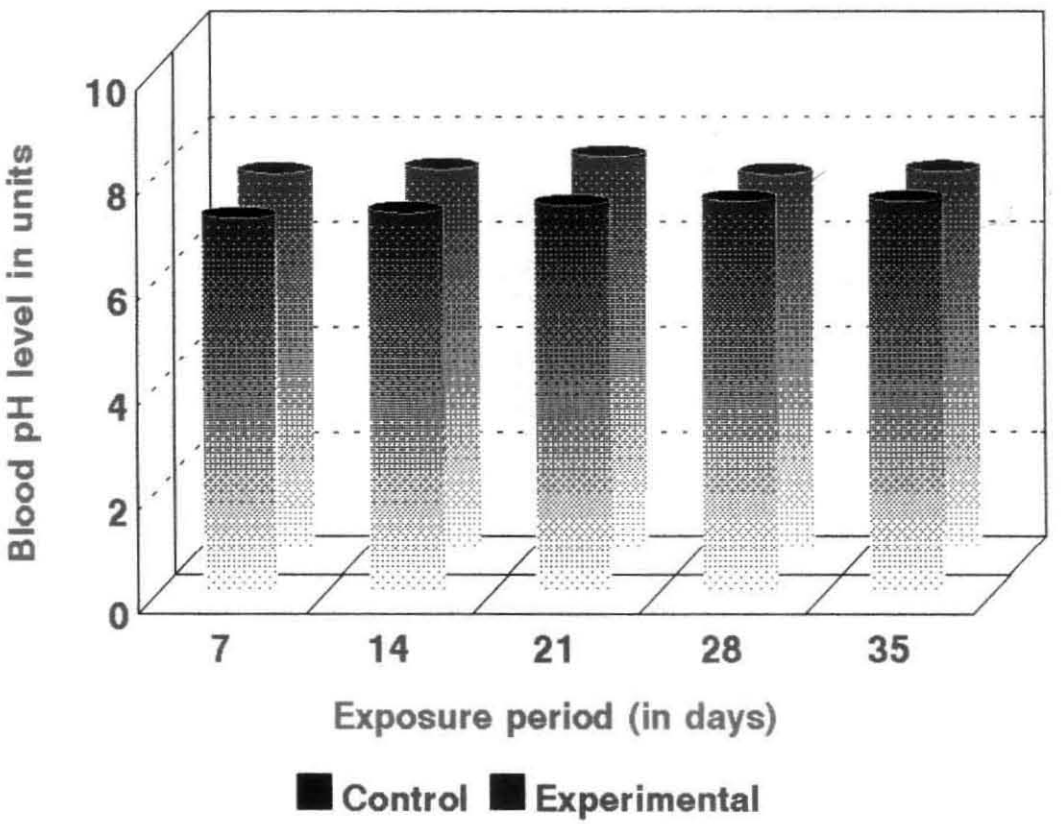
| Exposure period (in days) | Blood pH in units | | Per cent change |
|---|---|---|---|
| | Control | Experimental | |
| 7 | 7.309±0.006 | 7.409±0.009 | +1.37 |
| 14 | 7.400±0.036 | 7.487±0.039 | +1.18 |
| 21 | 7.541±0.034 | 7.728±0.061 | +2.48 |
| 28 | 7.614±0.108 | 7.380±0.064 | -3.07 |
| 35 | 7.614±0.112 | 7.452±0.103 | -0.95 |

Values are mean ± S . E . of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 8. Blood pH of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Blood pH of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

8). After 21st day, it however, declined giving a reduction of 3.07 per cent than the reference value at the end of 28th day. Again, pH level increased slightly at the end of 35th day exhibiting a per cent decline of 0.95 per cent from control values.

Table 13 presents the data on blood pH level when analysed by DMRT during sublethal aluminium toxicity. Blood pH was on par in the experimental group when compared with that of the control except on 28th day, which varied significantly.

When control group alone was tested 7th and 14th day, 14th and 21st day, 21st, 28th and 35th day were on par with each other, the rest of combination being significantly different from each other. In the case of sublethal treatment of aluminium, 7th, 14th, 28th and 35th day treatments were on par with each other, but 7th and 14th, 28th days varied significantly from that of 21st day. On the other hand 35th day was on par with 21st day.

Analysis of period means, irrespective of the treatments revealed that 7th, 14th and 28th day, 14th, 28th and 35th day and 21st, 28th and 35th days were on par with each other. The rest of the combinations being significantly different from each other. Analysis of treatments irrespective of the period, blood pH did not vary significantly from that of the control. Thus, analysis shows that pH level in the experimental group did not vary from the control during sublethal treatment.

Blood $HCO_3^-$ level increased initially giving 30.45 and 30.0 per cent after 7th and 14th day treatment respectively (Table 14 and Fig. 9) which then declined as the

Table 13 : DMRT table for blood pH in ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $7.309^{Aa}$ | $7.409^{Aa}$ | $7.359^{I}$ |
| 14 | $7.400^{ABa}$ | $7.487^{Aa}$ | $7.444^{I,II}$ |
| 21 | $7.541^{BCa}$ | $7.728^{Ba}$ | $7.635^{III}$ |
| 28 | $7.614^{Ca}$ | $7.380^{Ab}$ | $7.497^{I, II, III}$ |
| 35 | $7.614^{Ca}$ | $7.452^{ABa}$ | $7.578^{II, III}$ |
| Mean | $7.496^{\alpha}$ | $7.509^{\alpha}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at F <0.05
CV = 2.035 %

Table 14 : Bicarbonate level in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

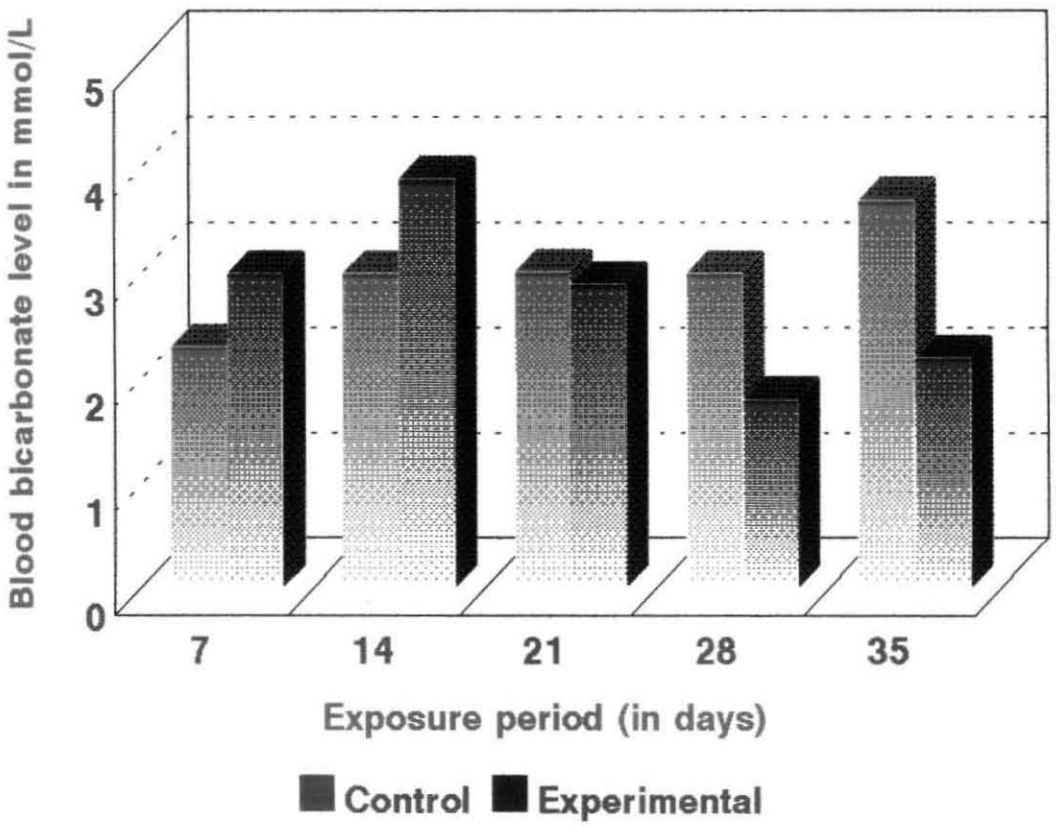
| Exposure period (in days) | Blood bicarbonate level in mmol/L | | Per cent change |
|---|---|---|---|
| | Control | Experimental | |
| 7 | 2.300±0.270 | 3.000±0.962 | +30.45 |
| 14 | 3.000±0.707 | 3.900±0.285 | +30.00 |
| 21 | 3.020±0.327 | 2.900±0.515 | -3.97 |
| 28 | 3.000±0.790 | 1.800±0.257 | -40.00 |
| 35 | 3.700±0.487 | 2.200±0.442 | -40.54 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 9. Bicarbonate levels in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Bicarbonate levels in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

exposure period was extended giving a per cent decline of 40.54 at the end of 35th day.

When the data were analysed by DMRT, blood $HCO^-_3$ level was found to be on par with control and experimental group at the close of 7th day. A similar trend was observed for all the exposure periods. Analysis of control group alone, for varying exposure periods revealed that $HCO_3^-$ level was on par in all exposure periods (Table 15). A similar trend was noted in the treatment group also except on 14th day. Analysis of period means irrespective of the treatment also exhibited a similar trend. When treatment groups were analysed irrespective of the treatment period showed that $HCO_3^-$ level was on par in both the treatment groups. Analysis altogether indicates that blood $HCO^-_3$ level did not vary significantly during sublethal aluminium exposed groups.

Table 15 : DMRT table for blood bicarbonate in ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $2.300^{Aa}$ | $3.000^{Aa}$ | $2.650^{I}$ |
| 14 | $3.000^{Aa}$ | $3.900^{Ba}$ | $3.450^{I}$ |
| 21 | $3.020^{Aa}$ | $2.900^{Aa}$ | $2.960^{I}$ |
| 28 | $3.000^{Aa}$ | $1.800^{Aa}$ | $2.400^{I}$ |
| 35 | $3.700^{Aa}$ | $2.200^{Aa}$ | $2.950^{I}$ |
| Mean | $3.004^{\alpha}$ | $2.760^{\alpha}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at $F < 0.05$
CV = 43.017 %

# DISCUSSION

The regulation of cations like $Na^+$, $K^+$, $Ca^+$ and $Mg^{2+}$ are disrupted in aquatic animals under toxic conditions as reported by Bruin (1976). Alterations in the ionic balance of aquatic organisms can be due to stressor effects on the ion regulatory organs (Spronk ***et al.***, 1971; Gilles and Requeux, 1983) or internal and external sensory receptors involved with detection of changes in osmotic conditions (Inman and Lockwood, 1977; Rosseland and Staurnes, 1994) or on the endocrine system (Mazeaud ***et al.***, 1977; Harman ***et al.***, 1980), or on the metabolism (Greenway, 1979), or on the active transport processes (Inman and Lockwood, 1977; Jowett ***et al.***, 1981; Staurnes ***et al.***, 1984).

McDonald ***et al.*** (1989) stated that branchial ionoregulation is the most likely physiological target of surface active metals due to the involvement of variety of potential targets and toxic mechanisms. Further, the authors stated that the potential physiological effect of metals is on the ionic regulation of fish and it could be due to any of the following : 1. disruption of one or more of the electrolyte transport mechanisms 2. widening or blockade of the diffusion pathways and 3. interference with the surface binding of calcium with a concomitant effect on membrane stability.

Acute exposure of freshwater fish to metals usually results in decreased plasma ions levels (Lock ***et al.***, 1981). Several authors have pointed out that cadmium induced plasma ionic disturbances are apparently caused by impaired uptake and diffusional losses of ion ***via.,*** the gills and leading to hyponatremia, hypochlorenemia

and hyperkalemia (Larsson *et al.*, 1981; Eddy, 1982; Giles *et al.*, 1984; Pratap *et al.*, 1989).

According to Poleo and Muniz (1993) and Poleo (1995), an increased thickness of the diffusion barrier between water and plasma due to aluminium polymerisation and mucus clogging may reduce the availability of electrolytes for the transport molecules integrated in the gill epithelial membrane. This finds support that ion influx may be affected by an inflammation and edema of the transporting gill epithelia in response to surfacial aluminium build up in brown trout, ***Salmo trutta***, as reported by Karlsson-Norrgren *et al.* (1986a,b).

McDonald (1983a), McWilliams (1983), Chevalier *et al.* (1985), Marshall (1985), Freda *et al.* (1991) have suggested that both the displacement of membrane bound $Ca^{2+}$ with subsequent weakness of the tight junctions with deformations and lifting of the gill epithelium could have accelerated ion loss under Al stress. The above mechanism of aluminium in depressing plasma $Na^{+}$ and $Cl^{-}$ concentrations was supported by NeVille (1985), Dalziel *et al.* (1986), Goss and Wood, (1988), Wood *et al.* (1988a, b) and Vuorinen *et al.* (1990,1992).

Booth *et al.* (1988) and McDonald and Milligan (1988) have recorded ion loss of $Na^{+}$, $Cl^{-}$ in brook trout, ***Salvelinus fontinalis***, and ***Salmo gairdneri***, after aluminium exposure and they are of the opinion that net ion loss in fish may be due to combination of inhibition of active ion influx through the "chloride cells" and stimulation of passive efflux through the paracellular channels. The depression in cation concentration may be due to reduced influx and greater efflux or impairment of

membrane permeability by stressors (Sivaprasad Rao *et al.*, 1983; Subba Rao, 1987; Ramesh, 1995).

In the present study the loss of $Na^+$ and $Cl^-$ ions in acute and sublethal studies may be due to the impaired uptake and diffusional losses ***via***, gills in ***Cyprinus carpio*** var. ***communis*** by aluminium stress as suggested by the above authors. Larsson ***et al.*** (1976) opined that osmoregulatory failure might be a reason for decreased levels of major plasma electrolytes. McCarty and Houstan (1976) stated that the decrease in plasma electrolytes levels tended to couple with increase in tissue concentrations.

Caldwell (1977) noted that the inhibition of ATPase activity in gills was a manifestation of the impairment of the ionic balance and regulation. Staurnes ***et al.*** (1984) stated that ion influx in rainbow trout, ***Salmo gairdneri***, may result directly from inhibition of transport enzymes like $Na^+$ $K^+$ ATPase by aluminium in the gills. The decrease in chloride uptake by aluminium exposed salmonids may involve inhibition of $HCO3^-$ ATPase as opined by Battaram (1988). A similar mechanism ***ie.***, inhibition of ATPase activity by aluminium toxicant may be operating in the present study.

Neville and Campbell (1988) ~~stated~~ observed in rainbow trout, ***Oncorhynchus mykiss***, treated with aluminium at pH 5.5 to 5.0 showed moderate ion flux responses and a transition between the two major response mechanisms in fishes (respiratory and ionoregulatory disturbances). This bimodal physiological response was also observed by Neville (1985), Goss and Wood (1988),

Reader *et al.*, (1988), Wood *et al.*, (1988a), Playle *et al.*, (1989), Dietrich and Schlatter (1989b) and Poleo *et al.* (1994) in fish. A similar bimodal mechanism may be operating in the present study during acute aluminium exposure as indicated by moderate decrease in $Na^+$ and $Cl^-$ level in the plasma of ***Cyprinus carpio*** var. ***communis*** where the pH of the experimental water was reduced to 5.0±1 after addition of LC50 24 h concentration of aluminium sulphate.

In the present study, the loss of electrolytes in the acute and sublethal studies may be due to aluminium polymers on the gills reducing the availability of electrolytes for transport or due to the inhibition of active ion influx or stimulation of passive efflux through paracellular channels or due to inhibition of transporting enzymes.

Increased levels of ions in the plasma of fish in sublethal treatment may be attributed to an increased synthesis of transport proteins responsible for sodium uptake (McDonald and Milligan, 1988) or by increasing gill binding affinity for $Ca^+$ thereby offsetting the competitive binding of $Al^{3+}$ (Reid *et al.*, 1991) or by the proliferation of "Chloride cells" on the branchial epithelium of adult salmonids (Laurent and Hebibi, 1988).

Booth *et al.* (1988) and Wood *et al.* (1988c) have reported for $Na^+$ increase, that the responses of naive fish to acid aluminium exposure may be characterised as occurring in two phases 1. The 'shock phase', (0-48 hr) identified by massive efflux of sodium and chloride and inhibition of sodium uptake at the gill . 2.the recovery phase' where efflux is reduced despite continued inhibition of uptake.

In ~~consistence to this~~ accordance with the observation, in the present study during sublethal exposure, initially $Na^+$ level decreased after 1st week indicating 'shock phase' and then increased in the subsequent weeks exhibiting 'recovery phase'.

According to Heming and Blumhagen (1988), the observed $K^+$ ion elevation in the plasma of rainbow trout, ***Oncorhynchus mykiss***, during acid and aluminium exposure, suggests the deterioration of cell membranes with resultant leakage of cellular potassium into the blood plasma. Increase in the plasma $K^+$ may also be due to reduction in the extracellular space (Booth ***et al.***, 1982; Milligan and Wood, 1982; Witters, 1986) or decreased plasma volume (Playle ***et al.***, 1989). A similar situation in the present study may account for the increase in plasma $K^+$ level recalling the above observations.

In the present study, blood pH and bicarbonate levels decreased in acute treatment. However, no significant change was observed in the sublethal studies. One of the most important task for homeostatic regulation is the maintenance of a set point pH in the body fluids of an animal (Heisler, 1982; Cameron, 1976). Claiborne and Heisler (1984) and Wood ***et al.*** (1984) have indicated that the exchange rates of $Na^+$, $H^+$ ($NH_4$) and $Cl^-$, $HCO_3^-$, $(OH)^-$ are dynamically manipulated in an appropriate manner during acid-base disturbances to achieve internal pH compensation.

Davenport (1974) stated that acidosis is a ~~compound~~ condition of uptake "respiratory" and "metabolic" (ie., depression of blood pH and $HCO_3^-$ without rise in $PCO_2$) origin of $H^+$ in the blood. Further acidosis is enhanced at reduced

environmental pH by inhibiting $Na^+/H^+$ and $Cl^-/HCo_3^-$ exchanges and rapid entry of $H^+$ ion into the body fluids.

Bass and Heath (1977) suggested that the inhibition of oxygen uptake (hypoxia) in the gills brings a shift to anaerobic metabolism causing decline in blood pH. According to Sellers ***et al.*** (1975), zinc exposure inhibits gaseous exchange in the gills of fish, then concurrently arterial blood pH decreases, ***i.e.***, lactoacidosis occurs probably due to the accumulation of lactic acid and $CO_2$ in the blood. Aluminium caused decreases in blood pH, which was attributed to respiratory acidosis, due to $CO_2$ accumulation or to metabolic acidosis due to lactic acid accumulation as a result of anaerobic respiration in rainbow trout, ***Oncorhynchus mykiss***, during acid and aluminium exposures (Playle ***et al.***, 1989).

Low pH environments are likely to interfere with acid-base regulation through gills in two important ways (McDonald and Wood, 1981; Heisler, 1982). Firstly, such environment inhibits active ion transport disrupting $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchange and thus disabling rapid responses of fish to blood acid-base disturbances (Heisler, 1982). Although kidney remains quite competent in acid-base regulation at low pH (McDonald and Wood, 1981), it cannot fully replace the function of gills as it responds very slowly to acid challenge (Wood and Caldwell, 1978). Secondly, the gills can act as a major route for $H^+$ ion permeation of body fluids when external $H^+$ is elevated (McDonald, 1983b).

Surplus $H^+$ ion produced during aluminium hydrolysis or aluminium induced hypoxia resulting in anaerobic metabolism or impaired gaseous exchange due

to mucus barrier at the gill surface provoking lactic acid and $PCO_2$ accumulation and inhibition of acid-base relevant ion exchanges might be the probable causes of blood pH decline observed in the present study.

According to Staurnes ***et al.*** (1984, 1993) the decline of $HCO_3^-$ level in the plasma of ***Oncorhynchus mykiss*** was due to inhibition of carbonic anhydrase enzyme by aluminium. A similar reason was attributed to blood $HCO_3^-$ depletion in trouts due to nitrite exposure by Giano ***et al.*** (1984) and Williams and Eddy (1988). An alternative explanation offered by Houston and Mearow (1982) for the decline of bicarbonate was while maintaining plasma electroneutrality one or more anions including bicarbonate may fall.

# HEMATOLOGY

# INTRODUCTION

Blood constituents have been well established for many of the higher vertebrates as diagnostic tools in human and animal medicine (Wedemeyer and Chatterton, 1970). Changes in blood parameters are often quick to respond environmental or physiological alterations, further more they are easily measurable and provide an integrated measure of the physiological status of organisms (Tort and Torres, 1988). Ghazaly (1992) emphasized the need for the study on hematological and physiological variables because of their relationship with respiration (hemoglobin content and related parameters), defense mechanisms (leucocyte values) and energetics (metabolite levels).

Significant elevation or reduction in hematological values of fishes exposed to different environmental toxicants have been reported by many workers (Svobodova, 1982; Ruparelia ***et al.***, 1990; Dalela ***et al.***, 1991; Ghazaly, 1992; Folmar ***et al.***, 1993; Ramesh, 1995; Gupta ***et al.***, 1995). Christensen ***et al.*** (1972) stated that any kind of stress not resulting in gross changes and mortality produces certain changes in the fish blood characteristics. Iwama ***et al.*** (1986) suggested that changes in the hematology of fishes in responses to stressing agents are indicators of the stressful state in fish and they are useful in deciding when to initiate prophylactic measures in fish culture. They may also provide a method of assessing the physiological status of fish.

Changes in hematological parameters such as hemoglobin content, HCT, RBC and WBC count can be induced by incidental factors such as capture and sampling (Bouck and Ball, 1966; Lowe-Jinde and Nimmi, 1983) as well as chronic factors such as exposure to disease and environmental contaminants (Johanssen-Sjobeck and Larsson, 1978 ; Barham ***et al.***, 1980). Hematological techniques including measurements of hematocrit, erythrocyte and leucocyte counts have proved valuable for fishery biologists in assessing the health of fish (Blaxhall, 1972; Hickey, 1976) and in monitoring stress responses (Swift and Lloyd, 1974; Soivio and Oikari, 1976).

Several species of fish are susceptible to the deleterious effects of heavy metals as reflected in the blood changes including anemia (Johansson-Sjobeck and Larsson, 1978,1979), eosinophilia (Dawson, 1935), lymphocytosis (Gardner and Yevich, 1970) and alterations in erythrocyte morphology. Toxic metals generally bind more strongly than do the essential metals, so displacing them from their normal sites though they also exert toxic effects by binding to other sites; among these, aluminium has different chemical characteristics from the familiar toxic metals and prefers oxygen sites exclusively (Martin, 1986; McDonald and Martin, 1988; Hughes and Poole, 1989).

Goss and Wood (1988) stated that estimation of hemoglobin can be used as an index of anemia and fluid volume disturbance. The hemoglobin content in fish blood has been elevated due to metal exposure ***viz.***, cadmium to ***Tilapia zillii*** (Ghazaly, 1992), chromium to rainbow trout ***Salmo gairdneri*** (Vander Putte ***et al.***,

1982) and in freshwater fish, ***Barbus conchonius*** (Gill and Pant, 1987), copper to brown bullhead, ***Ictalurus nebulosus***, (Christensen ***et al.***, 1972), brook trout, ***Salvelinus fontinalis***, (McKim ***et al.***, 1970) and to ***Oreochromis mossambicus*** (Cyriac ***et al.***, 1989), zinc to ***Clarius lazera*** (Hilmy ***et al.***, 1987) and mercury to ***Oreochromis mossambicus*** (Cyriac ***et al.***, 1989).

Hemoglobin content was decreased due to metal exposure ***viz.***, by cadmium in ***Perca fluviatilis*** (Sjobeck ***et al*** , 1984), by lead in ***Salvelinus fontinalis*** (Christensen ***et al.***, 1977) and by mercury in ***Pseudopleuronectes americanus*** (Dawson, 1979) and in ***Morone saxatilis*** (Dawson, 1982). Calabrese ***et al.*** (1975) did not observe any statistically significant change in hemoglobin, in flounders ***Pleuronectis americanus*** when exposed to cadmium.

Aluminium exposure in acidic waters reduced the hemoglobin content in ***Salmo gairdneri*** (Goss and Wood, 1988) , in brook trout, ***Salvelinus fontinalis***, (Wood ***et al.***, 1988a) and in females of whitefish, ***Coregonus wartmanni***, (Vuorinen ***et al.***, 1990). Whereas, the hemoglobin concentration in the males of whitefish, ***Coregonus wartmanni***, was increased by aluminium exposure at low pH (Vuorinen ***et al.***, 1990). The studies of Witters ***et al.*** (1986) in rainbow trout, ***Oncorhynchus mykiss***, Wood ***et al.*** (1988a, b) in brook trout, ***Salvelinus fontinalis,*** Vuorinen and Vuorinen (1991) in white fish, ***Coregonus wartmanni***, and Vuorinen ***et al.*** (1992) in ***Perca fluviatilis*** have indicated that in acidic waters aluminium toxicity did not alter the hemoglobin content of blood.

The hematocrit (HCT) of blood is the ratio of the volume of erythrocytes to that of the whole blood. Decrease in HCT by cadmium exposure was recorded in tilapia, ***Oreochromis mossambicus***, (Ruparelia ***et al.***, 1990), in flounders (Larsson ***et al.***, 1976), in ***Salmo gairdneri*** (Haux and Larsson, 1984) and in ***Tilapia zillii*** (Ghazaly, 1992). Significant increase in HCT values in different fishes exposed to chromium (Vander Putte ***et al.***, 1982), copper (McKim ***et al.***, 1970; Christensen ***et al.***, 1972; Cyriac ***et al.***, 1989) , mercury (Cyriac ***et al.*** (1989) and Zinc (Hilmy ***et al.***, 1987) was observed.

Significant increases in HCT value ~~was~~ were observed, in rainbow trout ***Salmo gairdneri*** ( Goss and Wood,1988;Witters ***et al.***, 1986; Playle ***et al.***, 1989; Witters ***et al.***, 1990), in adult brook trout, ***Salvelinus fontinalis***, (Wood ***et al.***, 1988b), in white fish, ***Coregonus wartmanni***, (Vuorinen ***et al.***, 1990; Vuorinen and Vuorinen, 1991), and in perch, ***Perca fluviatilis***, (Vuorinen ***et al.***, 1992), when exposed to aluminium in acidic waters. On the other hand, HCT showed no significant difference in its value in long term acid plus aluminium exposure in ***Salvelinus fontinalis*** (Mount ***et al.***, 1988), or selenium (Hodson ***et al.***, 1980) and methyl mercury exposure (Bleau ***et al.***, 1996) in ***Oncorhynchus mykiss***.

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions (Natarajan, 1981; Sakthivel, 1988; Ahmed and Munshi, 1989; Kumar and Barthwal, 1991). Reduction of RBC count in fish blood after cadmium in ***Carassius auratus*** (Houston and Keen, 1984), in ***Tilapia zillii*** (Ruparelia, 1990) and lead poisoning in ***Barbus conchonius*** (Tewari ***et al.***, 1987) has been reported.

Paradoxically, acute exposure of ***Tilapia zillii*** to higher concentrations of cadmium increased the erythrocyte count (Ruparelia, 1990). Similarly metals ***viz.***, Cr, Ni and Cu elevated the number of erythrocytes in freshwater teleost ***Colisa fasciatus*** (Srivatsava ***et al.***, 1979) and cobalt exposure in teleosts (Frovolo, 1960). However, RBC count did not change in response to selenium (Hodson ***et al.***, 1980) and aluminium (Witters ***et al.***, 1986) exposure to rainbow trout ***Oncorhynchus mykiss***.

Variation in leucocyte count provide a more sensitive index of stress than do changes in erythrocyte abundance (Mcleay and Gorden, 1977). Changes in the leucocyte system manifest in the form of leucocytosis with heterophilia and lymphopenia, which are characteristic leucocyte responses in animals under stress. (Gromyz and Kalkowska ***et al.***, 1981; Szubartowska, 1983; Mandal and Lahiri, 1985; Sen ***et al.***, 1992).

Cadmium exposure decreased leucocyte count in ***Tilapia zillii*** (Ghazaly, 1992). Leucopenia is a general consequence of exposure to metals (Mishra and Srivastava, 1980) and stressors (Ellis, 1981). Leucocytosis had also been observed in ***Colisa fasciatus*** after chromium exposure (Srivastava ***et al.***, 1979) and in ***Scyliorhinus canicula*** after zinc treatment (Flos ***et al.***, 1987).

Erythrocyte indices such as Mean Cellular Hemoglobin Concentration (MCHC), Mean Cellular Hemoglobin (MCH) and Mean Cellular Volume (MCV) are calculated for determining the size, content and hemoglobin concentration of red cells; these indices have been useful in the morphologic characterization of anemia; they

may be calculated from the red cell count, hemoglobin concentration and hematocrit (Nelson and Morris, 1989).

MCHC value declined in ***Clarius batrachus*** exposed to lead (Nath and Banerjee, 1995) and in ***Channa punctatus*** treated with copper (Singh, 1995). Acute exposure to cadmium produced apparent decrease in MCV and MCH in fish ***Tilapia zillii*** (Ghazaly, 1992). MCV (mean cell volume) increase was reported by Nath and Banerjee (1995) in lead nitrate exposed airbreathing fish, ***Clarius batrachus***. Similar increase in MCV was reported in fish exposed to mercury by Panigrahi and Mishra (1978), Sastry and Sharma (1980) and Naidu ***et al.*** (1984). In general, MCHC value was lowered in fish due to aluminium exposure in acidic waters (Milligan and Wood, 1982; Goss and Wood, 1988; Wood ***et al.***, 1988a, Vuorinen ***et al.***, 1990; Vuorinen and Vuorinen, 1991).

The foregoing review of literature, reveals that there is a paucity of information on the effect of aluminium toxicity on the hematological parameters of fish in neutral pH. The aim of the present investigation is to study the impact of aluminium toxicity in neutral pH on the hematological parameters of fish ***Cyprinus carpio*** var. ***communis***.

# MATERIAL AND METHODS

## HEMATOLOGICAL STUDIES

To assess the hematological profile of the control and treated fish, hemoglobin, hematocrit levels and RBC and WBC counts were measured in the whole blood of ***Cyprinus carpio*** var. ***communis***. Erythrocyte indices of fish ***viz.***, MCHC (Mean Cell Hemoglobin concentration), MCH (Mean Cell Hemoglobin) and MCV (Mean Cell Volume) were also calculated.

## HEMOGLOBIN

Hemoglobin content was analysed by using ABL 300 acid base laboratory (Radiometer Copenhagen, Denmark).

### Principle

The ABL 300 Acid Base laboratory is a automated and computerized micro blood gas and acid- base balance analyzer which measures Hb by hemoglobin photometer and pH, $PCO_2$, $PO_2$ by their respective electrodes on blood samples and calculates $HCO_3$, $TCO_2$, ABE (Actual Base Excess), SBE (Standard Base Excess), SBC (Standard bicarbonate), SAT ($O_2$ saturation), $O_2CT$ (Oxygen content) from the measured values.

The hemoglobin photometer provides a monochromatic light source with a 505 nm green interference filter. A transmittance principle is used with the light passing through 2 lenses, the filter and another lens before reaching the cuvette. The hemoglobin measurement is completed in the first 10 sec. of the measuring

programme. When a calibration occurs, the print out reads Hb values. The Hb zero point calibration is performed on the red calibrating solution since this has no hemoglobin content.

The hemoglobin content is derived from Beers equation.

$$cHB = K_1 (\log I_o - \log I_t)$$

Where,

cHB = Hemoglobin content

$K_1$ = Constant

$I_o$ = Intensity of incident light

$I_t$ = Intensity of transmitted light

The S 1556 red calibrator solution (for high pH) is used as the calibrator solution and S4706 rinse solution for rinsing the cuvette is used. These solutions are provided by Radiometer Copenhagen, Denmark and all measurements and calibrations are carried out at 37°C $\pm$ 1°C.

**Procedure**

The ABL 300 Acid-Base Laboratory was setup to obtain the ready lamp (green) to glow to analyse hemoglobin content in the sample. The heparinised blood sample was then aspirated through the inlet stub using a catheter tube and pressing the aspirate button immediately. The sample was aspirated till the sample lamp (yellow) glowed. Then the tube was removed and the inlet flap was tightly closed. The total

sample needed for measurement is 85 µl. The hemoglobin content was measured within 10 sec. of the measuring programme *ie.*, 71 sec. and the result was displayed as the concentration of hemoglobin in g/dl.

**HEMATOCRIT (Packed cell volume)**

Hematocrit was estimated by microhematocrit (capillary) method by sodium heparinised microhematocrit capillaries (Rakta Mucaps, Top Exports Private Ltd., Bombay, India) as described by Nelson and Morris (1989) using RM 12C microcentrifuge (Remi Centrifuge, Remi Motors, Bombay, India) and a microhematocrit reader.

**Principle**

When anticoagulated whole blood is centrifuged at a constant speed, erythrocytes (RBC), which are heavier than white cells, platelets and plasma, settled at the bottom. This red cell column is called hematocrit or packed red cell volume which is expressed as fraction of the whole blood (level of plasma). In microhematocrit method, the anticoagulated blood is centrifuged in a sealed capillary tube and the volume of packed red cells and percentage of the whole blood (level of plasma) are determined by a special hematocrit reader.

**Procedure**

Two sodium heparinised capillary microhematocrit tubes each about 7 cm long with an uniform bore of about 1 mm were taken. Then the blood from control and experimental groups kept in their respective vials was filled upto 5 cm by

capillary attraction. The sucking end of each tube was sealed with modelling clay and the filled tubes were placed in the radial grooves of the microhematocrit centrifuge head with the sealed end away from the center and speed at 10,000 rpm for 5 min. using RM 12 C microcentrifuge. The tubes were then taken and the length of the whole column including plasma and that of the red cell column alone was measured in a millimeter rule of the microhematocrit reader. The concentration of the red cells was taken as the hematocrit value which is expressed in percentage.

**RBC COUNT**

Erythrocytes were counted by the method of Rusia and Sood (1992) using hemocytometer.

**Principle**

The blood specimen is diluted with red cell diluting fluid. It destroys the white cells, but allows the red cells to be counted in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of cells per cubic millimeter of whole blood.

**Procedure**

A clean RBC pipette was taken and blood was drawn upto the 0.5 mark. The tip of the pipette was wiped clean and dipped vertically into the red cell diluting fluid , which was then sucked upto mark 101. Then the tip of the pipette was closed with a finger and the contents were mixed thoroughly by shaking the pipette at right angles to its long axis. The red bead in the bulb helps for proper mixing .

The ruled part of the hemocytometer was focussed clearly. Then the first drop of the diluted blood in the pipette was discarded. By holding the pipette at an angle of 45°, the diluted blood was blown, into the counting chamber. The blood was drawn into the chamber by capillary action. Then the counting chamber was left for 3 minutes to allow the cells to settle down.

**Counting**

The slide was first examined under low power and then under high power magnification. The counting chamber of the hemocytometer has a central heavy ruled area of 1 sq. mm. This central area is the RBC counting chamber. It is divided in to 25 squares and each square is subdivided into 16 small squares. For the erythrocyte count, the cells lying within and those touching the right and upper margin of the four corner squares and the central square (80 small squares) were counted. The total number of erythrocytes per cubic millimeter of whole blood was then calculated.

**Calculation**

$$\text{Number of erythrocytes (million/cu.mm)} = \frac{\text{Number of erythrocytes counted} \times \text{Dilution of blood}}{\text{Area counted} \times \text{Depth of fluid}}$$

Dilution = 200
Area counted = 5 x 0.04 = 0.2 sq.mm
Depth of fluid = 0.1 mm

**LEUCOCYTE COUNT**

Leucocytes were counted by the method of Rusia and Sood (1992) using hemocytometer.

**Principle**

Blood is diluted with acid solution which removes the red cells by hemolysis and accentuates the nuclei of the white cells. Counting is done with a microscope under low power and knowing the volume of fluid examined and the dilution of the blood, the number of white cells per cubic millimeter in undiluted whole blood is calculated.

**Procedure**

Blood was drawn upto the 0.5 mark using a clean WBC pipette. Then the pipette was immediately kept in a watch glass containing WBC diluting fluid and it was drawn upto mark 101, taking care that no air bubble was included. The contents were mixed well by rotating the pipette between the palms of the hands. The white bead in the pipette helps for proper mixing of blood with the diluting fluid. The diluted blood was allowed to stand as such for 3 minutes for hemolysis of red cells to occur. Again the contents were mixed by rotating the pipette.

After discarding the first few drops of diluted blood, the counting chamber of the hemocytometer was charged with the fluid, making sure that no air bubbles were trapped between the coverslip and the chamber. The cells were allowed to settle down for a minute.

**Counting**

For the counting of leucocytes, the slide was examined under low power magnification of microscope. The Neubaeur's counting chamber is divided into two

counting areas which are ruled. Each counting chamber is divided into a total ruled area of 9 sq.mm. The area of each square is 1 sq.mm. The 1 sq.mm area at the 4 corners of the slide were used for the counting of leucocytes. The cells falling within the four corner squares were counted and the total number of leucocytes per cubic millimeter of whole blood was calculated.

**Calculation**

$$\text{Number of leucocytes (thousands/cu.mm)} = \frac{\text{Number of leucocytes counted x Dilution of blood}}{\text{Area counted x Depth of fluid}}$$

Dilution = 20

Area counted = 4 x 1 = 4 sq.mm

Depth of fluid = 0.1 mm

**1. MEAN CELL HEMOGLOBIN CONCENTRATION (MCHC)**

The MCHC is the average concentration of hemoglobin in a given volume of packed red cells. It is calculated from the hemoglobin concentration and the hematocrit.

$$\text{MCHC(g/dl)} = \frac{\text{Hb ( g /dl)}}{\text{HCT (\%)}} \times 100$$

**2. MEAN CELL HEMOGLOBIN (MCH):**

The MCH is the content (weight) of hemoglobin of the average red cell. It is calculated from the hemoglobin concentration and the red cell count

$$\text{MCH (picograms)} = \frac{\text{Hb ( g /dl) x 10}}{\text{RBC (million/cu.mm)} \times 10^6}$$

**3. MEAN CELL VOLUME (MCV):**

The MCV is the average volume of red cells and is calculated from the hematocrit (HCT) and the red cell count (RBC).

$$\text{MCV (cubic micra)} = \frac{\text{HCT (\%) x 10}}{\text{RBC (millions/cu. mm)} \times 10^6}$$

# RESULTS

Changes in hematological profiles of fish, ***Cyprinus carpio*** var. ***communis*** during acute aluminium exposure are given in Table 16 and Fig. 10. Hemoglobin content and hematocrit value decreased from the control level by 11.39% and 7.55%, respectively. However, their decrease was not significant. Erythrocyte and leucocyte counts were altered significantly from the control value by registering 15.38% decrease and 24.99% increase, respectively.

Erythrocyte indices such as MCHC declined in the experimental groups when compared to that of control giving a per cent decrease of 3.85%, while MCH and MCV increased than that of control by 5.72% and 9.60%, respectively. However, statistical analysis revealed that these changes were not significant.

Table 17 and Fig. 11 give the data on changes in hemoglobin content of the fish blood during sublethal aluminium treatment. At the end of 7th day a pronounced decline in hemoglobin content (57.14%) was observed in the experimental fish which gradually recovered during the subsequent exposure periods showing a decrease of 43.18%, 34.75%, 29.57% and 21.88% after 14th, 21st, 28th and 35th day, respectively.

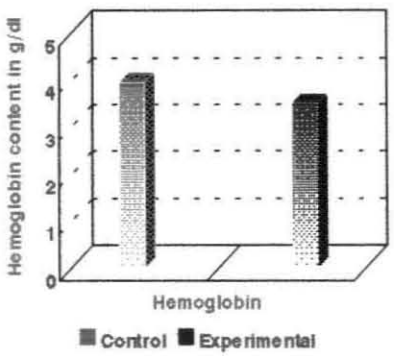
DMRT analysis between control and treatment for all the exposure period showed that the hemoglobin content varied significantly in the treatment group than that of the control (Table 18).

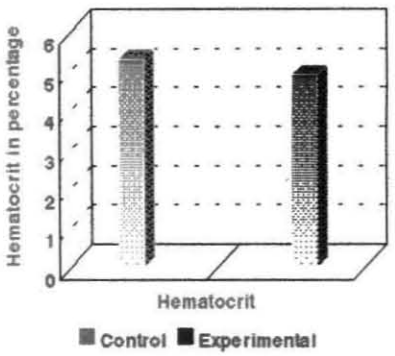
Table 16 : Changes in the hematological profiles of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.

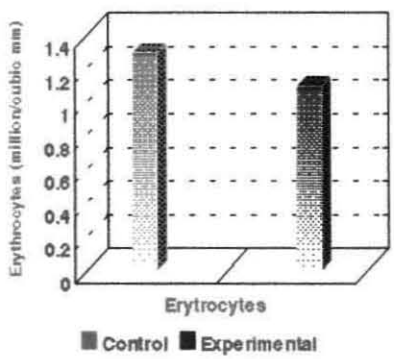
| Parameters | Control | Experimental | Per cent change | Calculated ' t' |
|---|---|---|---|---|
| Hemoglobin (g/dl) | 3.950 ± 0.1432 | 3.500 ± 0.1581 | - 11.39 | 2.1096 |
| Hematocrit (%) | 5.300 ± 0.2550 | 4.900 ± 0.2915 | - 7.55 | 1.0328 |
| Erythrocytes (million / cubic mm) | 1.300 ± 0.0241 | 1.100 ± 0.0606 | - 15.38 | 3.0679 * |
| Leucocytes (thousands / cubic mm) | 20.150 ± 791.7013 | 25.180 ± 574.9259 | +24.99 | 5.1470 * |
| MCHC (g / dl) | 75.440 ± 5.6626 | 72.534 ± 5.574 | -3.85 | 0.3657 |
| MCH (picograms) | 30.408 ± 1.1451 | 32.148 ± 2.0365 | + 5.72 | 0.7447 |
| MCV (cubic micra) | 40.740 ± 1.4389 | 44.650 ± 1.6542 | + 9.60 | 1.7843 |

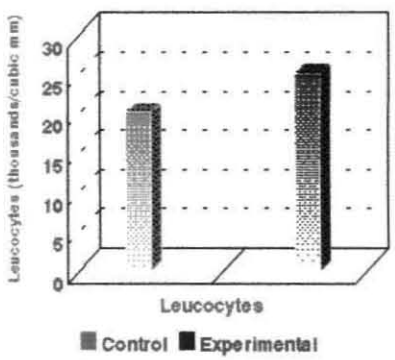
Values are means ± S . E . of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control
Values are significant at 5% level
Degrees of freedom at $8_{t\,0.05}$ = 2.306.

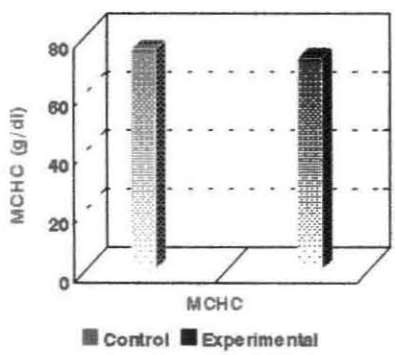
Fig. 10. Changes in the hematological profiles of ***Cyprinus carpio*** var. ***communis*** exposed to 24 h LC 50 concentration of aluminium sulphate.

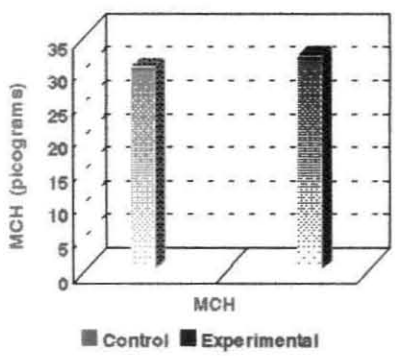


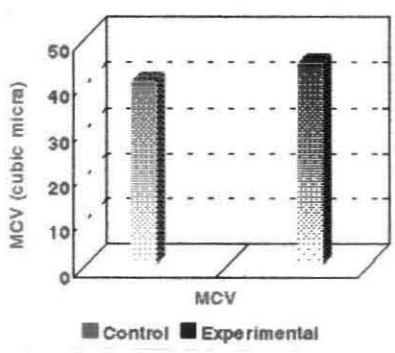




Changes in the hematological profiles of *Cyprinus carpio* var. *communis* exposed to 24h $LC_{50}$ concentration of aluminium sulphate

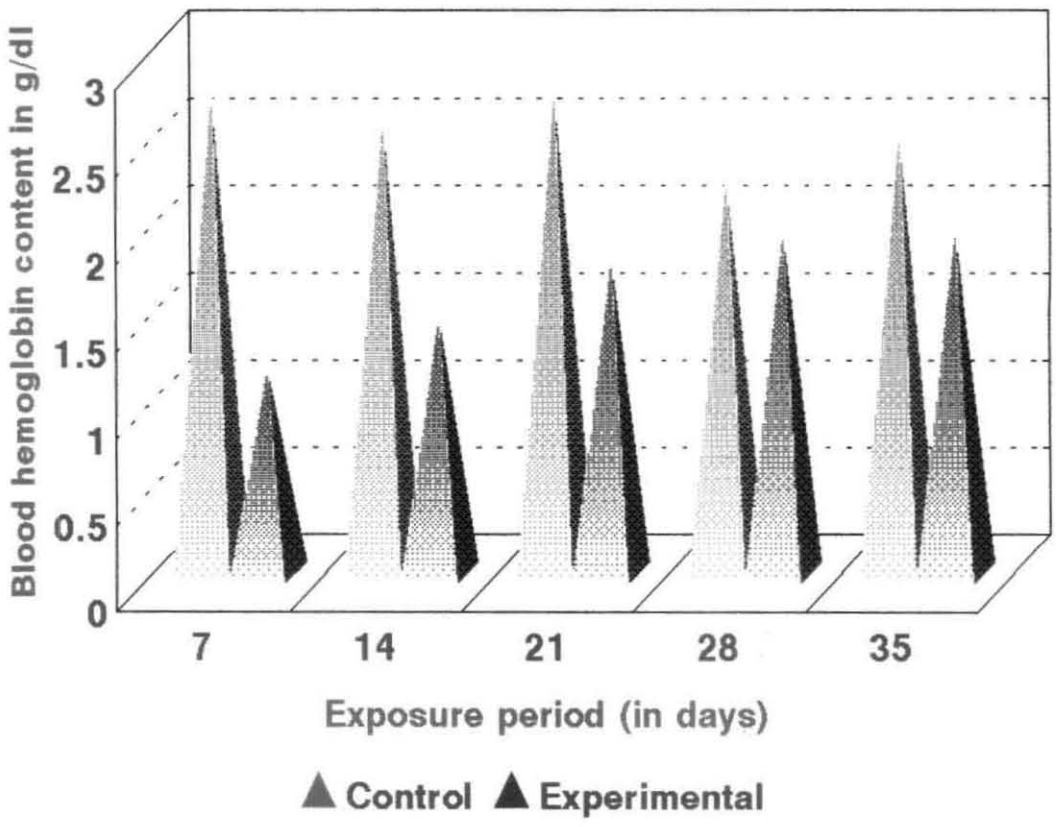
Table 17 : Hemoglobin content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Blood hemoglobin content in g/dl | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 2.800 ± 0.164 | 1.200 ± 0.100 | -57.14 |
| 14 | 2.640 ± 0.600 | 1.500 ± 0.032 | -43.18 |
| 21 | 2.820 ± 0.132 | 1.840 ± 0.081 | -34.75 |
| 28 | 2.300 ± 0.130 | 1.620 2.000 ± 0.037 | -29.57 |
| 35 | 2.560 ± 0.051 | 2.000 ± 0.032 | -21.88 |

Values are means ± S. E. of five individual observations

'-' Denotes per cent decrease over control

Fig. 11. Hemoglobin content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Hemoglobin content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 18 : DMRT table for hemoglobin content in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium sulphate toxicity for varying periods.

| Exposure period (in days ) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $2.800^{Ca}$ | $1.200^{Ab}$ | $2.000^{I}$ |
| 14 | $2.640^{BCa}$ | $1.500^{Bb}$ | $2.070^{I}$ |
| 21 | $2.820^{Ca}$ | $1.840^{CDb}$ | $2.330^{II}$ |
| 28 | $2.300^{Aa}$ | $1.620^{BCb}$ | $1.960^{I}$ |
| 35 | $2.560^{ABa}$ | $2.000^{Db}$ | $2.280^{II}$ |
| Mean | $2.624^{\alpha}$ | $1.632^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 9.835 %

When the control group alone was tested 7th and 28th, 14th and 28th, 21st and 28th, 7th and 35th days were significantly different whereas 7th and 14th, 14th and 21st, 14th and 35th, 28th and 35th day were on par with each other. When treatment group alone was tested, 7th day varied significantly from all other exposure periods, whereas 14th and 28th day, 21st and 28th day, 21st and 35th day were on par with each other and they varied from the rest of the combinations.

When period means were analysed, 7th, 14th and 28th days, 21st and 35th days were not significantly different among each other but they varied with the rest of their combinations. When treatment means were analysed, both the control and treatment varied significantly among each other, thus showing that sublethal aluminium impart toxic effect on hemoglobin level in fish.

Exposure of fish to sublethal aluminium sulphate resulted in a 11.63% increase in the hematocrit value of fish at the end of 7th day (Table 19, Fig. 12). However after 7th day, the hematocrit value declined giving a per cent decline of 30.00%, but at the end of the 21st day, the hematocrit reached a value equal to that of the control . At the end of the 28th day the hematocrit value again decreased showing 17.65%. On contrary, at the end of the 35th day, hematocrit value increased by 50% than that of the control.

Statistical analysis of hematocrit changes in the blood of ***Cyprinus carpio*** var. ***communis*** by DMRT during sublethal aluminium treatment, is given in Table 20. When both the control and treatment groups were analysed for

Table 19 : Hematocrit values of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium sulphate toxicity for varying periods.

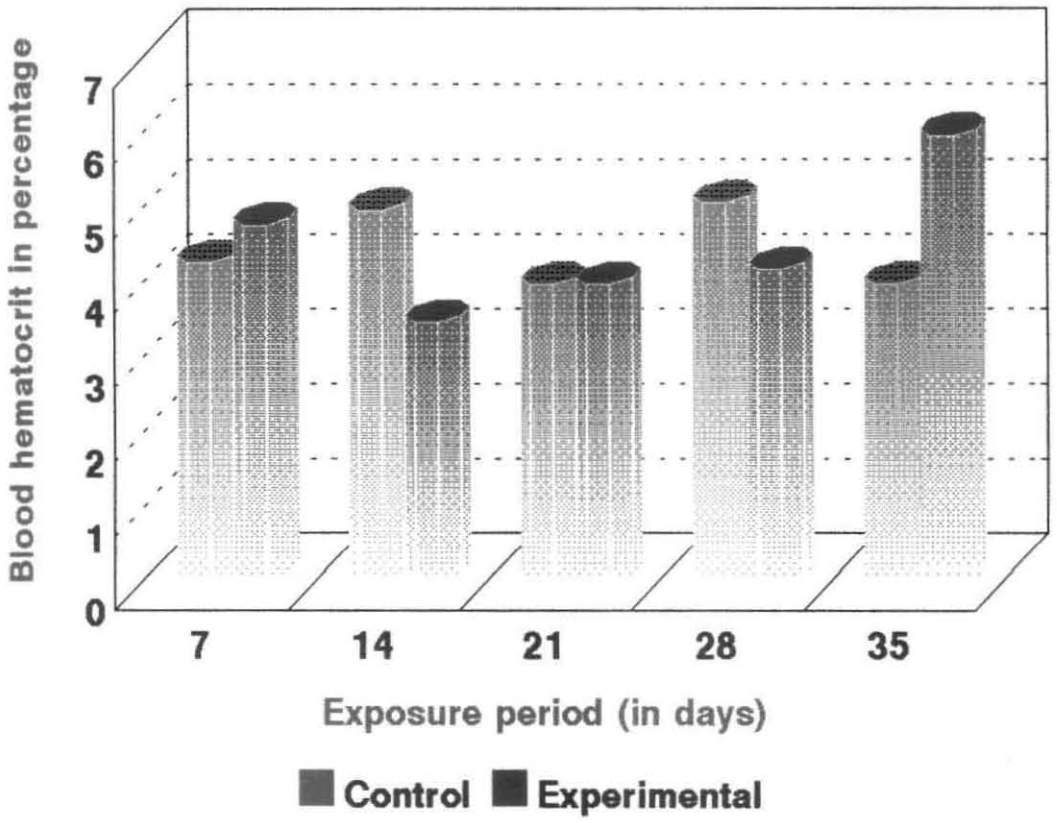
| Exposure period (in days) | Blood hematocrit (%) | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 4.300 ± 0.122 | 4.800 ± 0.255 | +11.63 |
| 14 | 5.000 ± 0.354 | 3.500 ± 0.354 | -30.00 |
| 21 | 4.000 ± 0.354 | 4.000 ± 0.354 | -0.00 |
| 28 | 5.100 ± 0.100 | 4.200 ± 0.122 | -17.65 |
| 35 | 4.000 ± 0.354 | 6.000 ± 0.158 | +50.00 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 12. Hematocrit values of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Hematocrit values of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 20 : DMRT table for blood hematocrit value of ***Cyprinus carpio*** var. ***communis*** to aluminium sulphate exposure for varying periods .

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $4.300^{ABa}$ | $4.800^{Bb}$ | $4.550^{I,II,III}$ |
| 14 | $5.000^{Ba}$ | $3.500^{Ab}$ | $4.250^{I,II}$ |
| 21 | $4.000^{Aa}$ | $4.000^{Aa}$ | $4.000^{I}$ |
| 28 | $5.100^{Ba}$ | $4.200^{ABb}$ | $4.650^{II, III}$ |
| 35 | $4.000^{Aa}$ | $6.000^{Cb}$ | $5.000^{III}$ |
| Mean | $4.480^{\alpha}$ | $4.500^{\alpha}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 13.684 %

varying exposure periods, the changes in hematocrit value were significant during all the exposure periods except 21st day which was on par.

Analysis of control group alone for varying periods showed the 7th, 21st and 35th day, 7th, 14th, and 28th day were on par with each other, while 14th and 21st, 14th and 35th, 21st and 28th, 28th and 35th were significantly different.

Analysis of treatment group alone for varying periods showed that 7th and 28th, 14th, 21st and 28th day were on par with each other. On the other hand 35th day varied significantly from the rest of the exposure periods.

Analysis of period means irrespective of the treatment indicated that 7th, 14th and 21st, 7th, 14th and 28th and 7th, 28th and 35th days were on par with each other, while the rest of the combinations being significantly different from each other. Analysis of treatment means irrespective of the exposure period revealed that both the groups ***viz***., control and test were on par with each other.

Red blood cell count of the blood samples of ***Cyprinus carpio*** var. ***communis*** after 7th day treatment was reduced drastically by 45.69%. However, there was a gradual recovery during subsequent exposure periods giving a per cent decline of 38.20%, 30.76%, 27.25% and 21.54% after 14th, 21st, 28th and 35th day, respectively (Table 21 and Fig. 13).

Table 22 gives the data on DMRT analysis of red blood cell content in the fish blood. When the treatment and control groups were analysed for varying periods of exposure both the groups varied significantly from each other. When

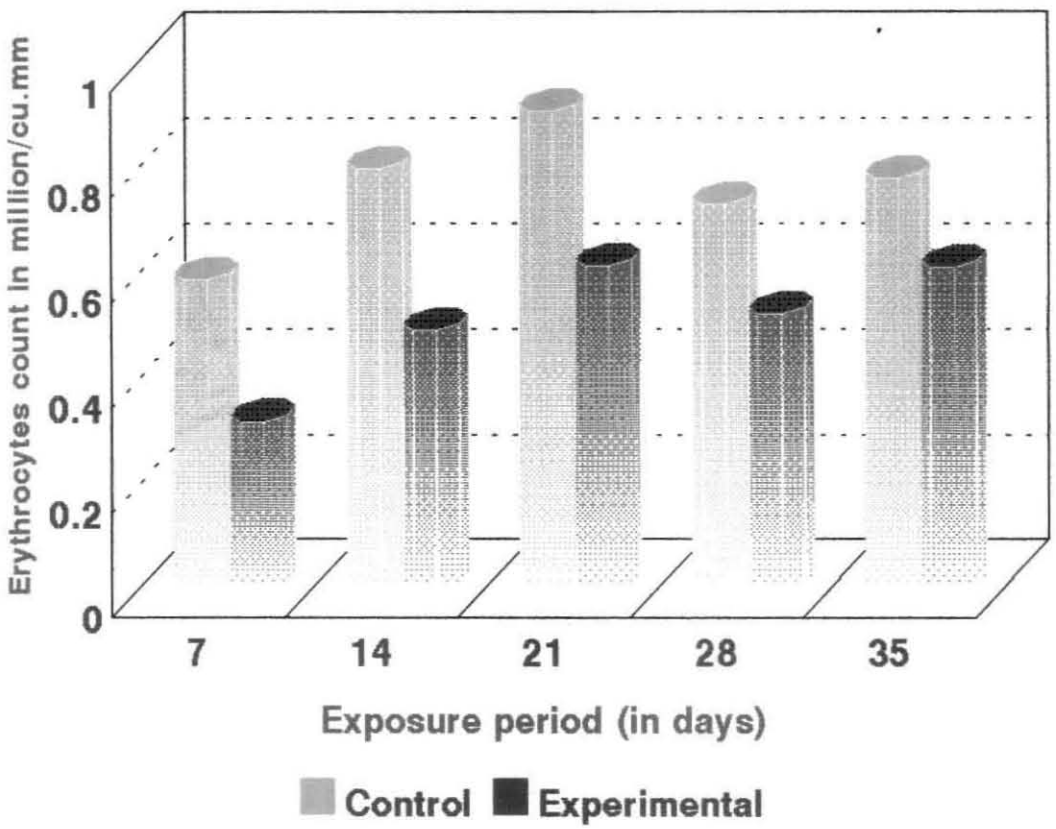
Table 21 : Erythrocytes count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Erythrocytes counts in million/cu.mm | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 0.591 ± 0.017 | 0.321 ± 0.013 | -45.69 |
| 14 | 0.801 ± 0.025 | 0.495 ± 0.030 | -38.20 |
| 21 | 0.910 ± 0.016 | 0.630 ± 0.032 | -30.76 |
| 28 | 0.722 ± 0.109 | 0.526 ± 0.026 | -27.25 |
| 35 | 0.783 ± 0.032 | 0.614 ± 0.286 | -21.54 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

Fig. 13. Erythrocytes count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Erythrocytes count in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 22 : DMRT table for erythrocyte count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $0.5918^{Aa}$ | $0.3210^{Ab}$ | $0.456^{I}$ |
| 14 | $0.8010^{Ca}$ | $0.4950^{Bb}$ | $0.648^{II, III}$ |
| 21 | $0.9100^{Da}$ | $0.6300^{Db}$ | $0.770^{IV}$ |
| 28 | $0.7226^{Ba}$ | $0.5260^{Bb}$ | $0.624^{II}$ |
| 35 | $0.7832^{BCa}$ | $0.6140^{CDb}$ | $0.699^{III}$ |
| Mean | $0.7617^{\alpha}$ | $0.5172^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 9.125 %

control groups alone were tested 14th and 35th, 28th and 35th days were on par with each other. The rest of the periods varied significantly among themselves. When treatment groups alone were tested 14th and 28th day, 21st and 35th day were on par with each other. The rest of the combinations varied from the above mentioned periods and also among themselves.

When period means were analysed irrespective of the treatments 14th and 28th, 14th and 35th day were on par with each other while 7th and 21st day varied among themselves and varied from that of 14th, 28th and 35th day. Analysis of treatment means showed that the experimental groups varied from the control depicting the aluminium impact on the red blood cell count of the blood.

Table 23 and Fig. 14 present the data on the changes in WBC count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate. The WBC count increased than that of the control by 80.97% on 7th day treatment and further increased on 14th and 21st day giving a value of 133.33 % and 130.23%, respectively. However, the WBC count declined gradually in the following weeks (28th and 35th day) showing 82.98% and 50.49% increase from the control, respectively.

DMRT analysis between the control and treatment for all the exposures period showed that the WBC count of fish blood varied significantly from that of the control (Table 24).

When the control group alone was tested 7th, 14th, 21st and 35th days were on par with other, while 28th day varied significantly from all other exposure

Table 23 : Leucocyte count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

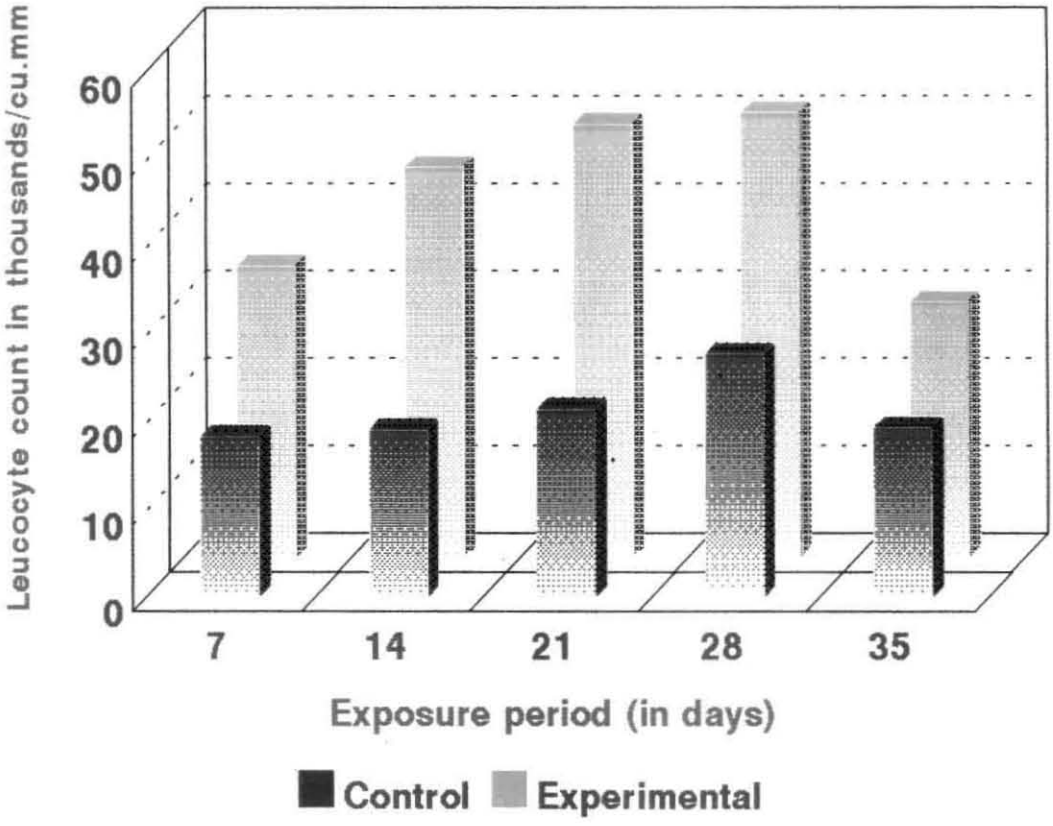
| | Leucocyte counts in thousands/cu.mm | | |
|---|---|---|---|
| Exposure period (in days) | Control | Treatment | Per cent change |
| 7 | 18.500± 0.802 | 33.480±1.317 | +80.97 |
| 14 | 19.200± 0.664 | 44.800±1.037 | +133.33 |
| 21 | 21.600± 1.977 | 49.730±1.176 | +130.23 |
| 28 | 27.900± 1.234 | 51.051±0.806 | +82.98 |
| 35 | 19.510± 0.654 | 29.360± 0.758 | +50.49 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 14. Leucocytes count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




Leucocytes count in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 24 : DMRT table for the leucocytes count from the blood of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $18.500^{Aa}$ | $33.480^{Bb}$ | $25.990^{I}$ |
| 14 | $19.200^{Aa}$ | $44.800^{Cb}$ | $32.000^{II}$ |
| 21 | $21.600^{Aa}$ | $49.730^{Db}$ | $35.665^{III}$ |
| 28 | $27.900^{Ba}$ | $51.050^{Eb}$ | $39.475^{IV}$ |
| 35 | $19.510^{Aa}$ | $29.360^{Ab}$ | $24.435^{I}$ |
| Mean | $21.342^{\alpha}$ | $41.684^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at $F < 0.05$
CV = 8.474 %

periods. When treatment group alone was tested, all the treated periods varied among each other.

When period means were analysed 7th and 35th day were on par with each other, and varied significantly from the other period means, whereas, 14th and 21st day and 28th day varied significantly among themselves. Analysis of treatment means irrespective of the exposure period revealed that the treatment groups varied significantly from that of the control indicating sublethal stress of aluminium causing changes in the WBC count of fish blood.

The changes occurred due to the sublethal aluminium exposure in the MCHC value is given in Table 25 and Fig. 15. MCHC value declined by 60.99% after 7th day exposure and in the subsequent weeks the per cent decline was reduced giving a value of 16.38%, 34.54% and 14.37% on 14th, 21st and 28th day, respectively. However, after the 35th day the per cent decline of the MCHC value again ~~raised~~ increased showing 49.41% ~~than~~ declined to relative to ~~that of~~ the control.

DMRT analysis revealed that MCHC value varied significantly in the treatment groups than that of the control after 7th day (Table 26). A similar trend was observed for 21st and 35th day. On the other hand at the end of 14th and 28th day both the control and treatment groups were on par with each other.

When control groups were analysed for varying periods 7th, 14th and 35th, 14th and 28th, 21st and 35th day were on par with each other. The rest of the combinations varied significantly among each other. When treatment groups alone were analysed, 7th, 28th and 35th day, 14th and 28th day and 14th and 21st day were

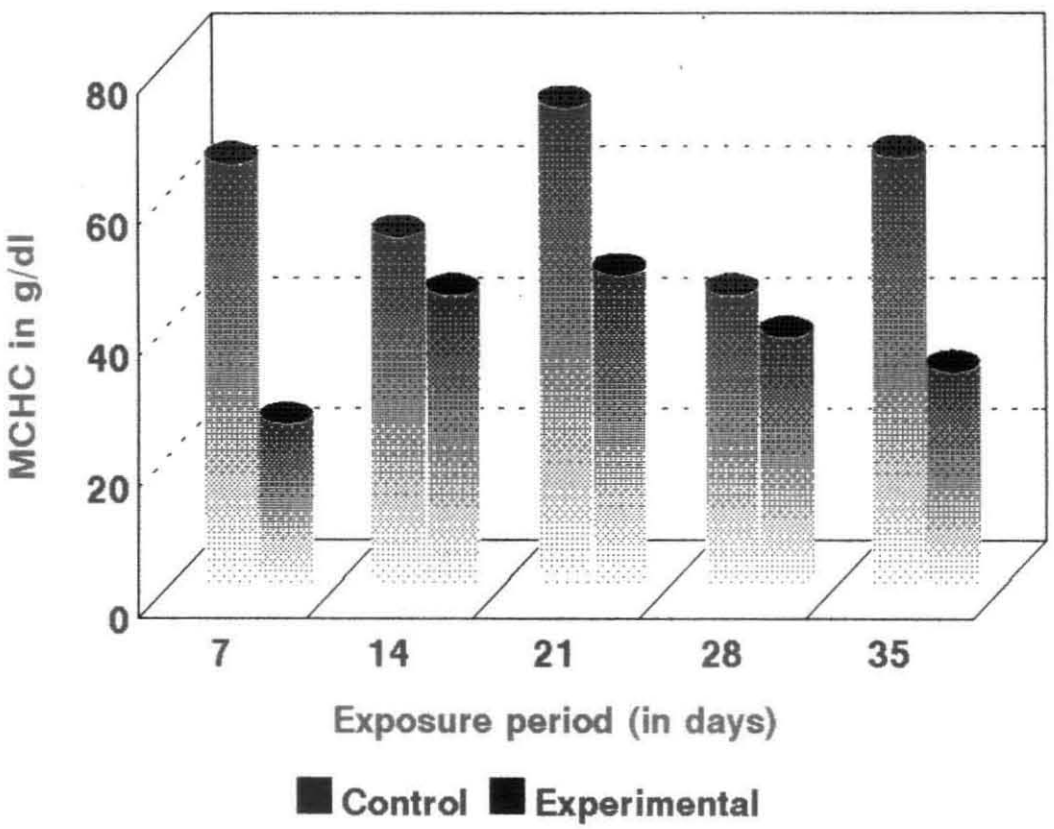
Table 25 : MCHC value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| | Mean Cellular Haemoglobin Concentration in g/dl | | |
|---|---|---|---|
| Exposure period (in days) | Control | Treatment | Per cent change |
| 7 | 65.056 ± 3.0482 | 25.378 ± 2.6308 | -60.99 |
| 14 | 53.926 ± 4.161 | 45.094 ± 5.656 | -16.38 |
| 21 | 73.524 ± 8.866 | 48.130 ± 6.301 | -34.54 |
| 28 | 45.127 ± 2.586 | 38.667 ± 2.650 | -14.37 |
| 35 | 65.990 ± 5.808 | 33.383 ± 0.815 | -49.41 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

Fig. 15. MCHC value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




MCHC value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 26 : DMRT table for Mean Cellular Haemoglobin Concentration (MCHC) value of the blood of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $65.056^{Ba}$ | $25.378^{Ab}$ | $45.217^{I}$ |
| 14 | $53.926^{ABa}$ | $45.094^{BCa}$ | $49.510^{I}$ |
| 21 | $73.524^{Ca}$ | $48.130^{Cb}$ | $60.827^{II}$ |
| 28 | $45.127^{Aa}$ | $38.667^{ABa}$ | $41.897^{I}$ |
| 35 | $65.990^{BCa}$ | $33.383^{Ab}$ | $49.687^{I}$ |
| Mean | $60.725^{\alpha}$ | $38.130^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 21.522 %

on par with each other. 7th and 14th, 7th and 21st, 14th and 35th and 21st and 35th, 21st and 28th day varied significantly among each other.

When period means were analysed irrespective of the treatments, only 21st day varied from the rest of the exposure periods, which were on par with each other. when treatment means were analysed irrespective of the exposure period, the means of MCHC value of aluminium exposed fish varied significantly than that of the control.

Table 27 and Fig. 16 illustrate the changes occurring in the MCH value due to aluminium stress in fish blood. The MCH value decreased than that of the control after 7th day recording 21.65%. During following periods, MCH value reduction was very low registering 7.28%, 2.18% and 2.54%, on the 14th, 21st and 28th day, respectively. Whereas on 35th day, MCH value increased than that of the control by 9.64%.

DMRT analysis of MCH showed that on 7th day, both the control and treatment groups varied significantly among themselves (Table 28). While in all other exposure periods they were on par with each other.

Analysis of control group alone depicted that 7th day was significant from that of 14th, 21st, 28th and 35th day which were on par with each other. When treatment groups were analysed 14th, 21st and 28th day, 14th, 28th and 35th day, 7th, 28th and 35th day were on par with each other, whereas 7th and 14th day, 7th and 21st day, 21st and 35th days varied significantly among themselves.

Table 27 : MCH value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

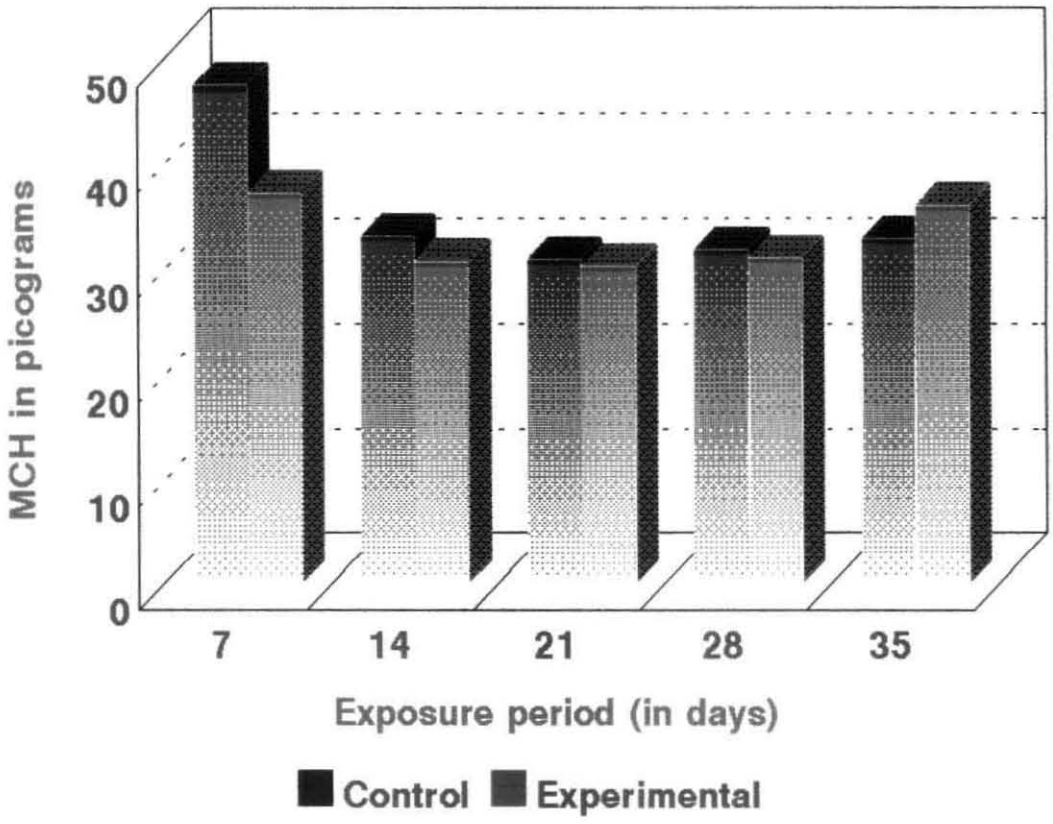
| | Mean Cellular Hemoglobin value in picograms | | |
|---|---|---|---|
| Exposure period (in days) | Control | Treatment | Per cent change |
| 7 | 47.572 ± 3.385 | 37.271 ± 2.373 | -21.65 |
| 14 | 33.151 ± 1.666 | 30.737 ± 1.930 | -7.28 |
| 21 | 30.972 ± 1.229 | 30.296 ± 2.272 | -2.18 |
| 28 | 31.909 ± 2.090 | 31.110 ± 1.723 | -2.54 |
| 35 | 32.904 ± 1.457 | 36.075 ± 1.040 | +9.64 |

Values are mean ± S. E. of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 16. MCH value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




MCH value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 28 : DMRT table for MCH value of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $47.572^{Ba}$ | $37.271^{Cb}$ | $42.419^{II}$ |
| 14 | $33.151^{Aa}$ | $30.737^{ABa}$ | $31.944^{I}$ |
| 21 | $30.972^{Aa}$ | $30.296^{Aa}$ | $30.634^{I}$ |
| 28 | $31.909^{Aa}$ | $31.110^{ABCa}$ | $31.509^{I}$ |
| 35 | $32.904^{Aa}$ | $36.075^{Bca}$ | $34.490^{I}$ |
| Mean | $35.300^{\alpha}$ | $33.098^{\alpha}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 13.207 %

When period means were analysed irrespective of the treatment, a parallel trend was noticed from the control, when the treatment means were analysed irrespective of the exposure period both the control and treatment groups were on par with each other.

Table 29 and Fig. 17 give the data on changes in the treatment groups with regard to MCV value in fish. There was a conspicuous per cent increase of 105.53% in the MCV value in aluminium treated fishes after 7th day. However, the increase in MCV value decreased in the following weeks giving 16.09%, 46.76% and 14.09% on 14th, 21st and 28th day, respectively. However, on 35th day, the per cent change in MCV value again rose to 94.71% relative to the control.

Table 30 gives the data on the analysis of MCV value of blood of ***Cyprinus carpio*** var. ***communis*** by DMRT. When both the control and treatment groups were analysed for varying exposure period, both the groups varied significantly among each other.

When the control groups alone was tested for varying exposure periods, 7th, 14th day and 28th day and 21st and 35th day were on par with each other but they varied in the rest of the combinations. Similarly when the treatment groups were analysed 7th and 14th day, 14th and 21st day were on par with each other, but varied significantly from that of 7th, 28th and 35th day, which also varied among themselves.

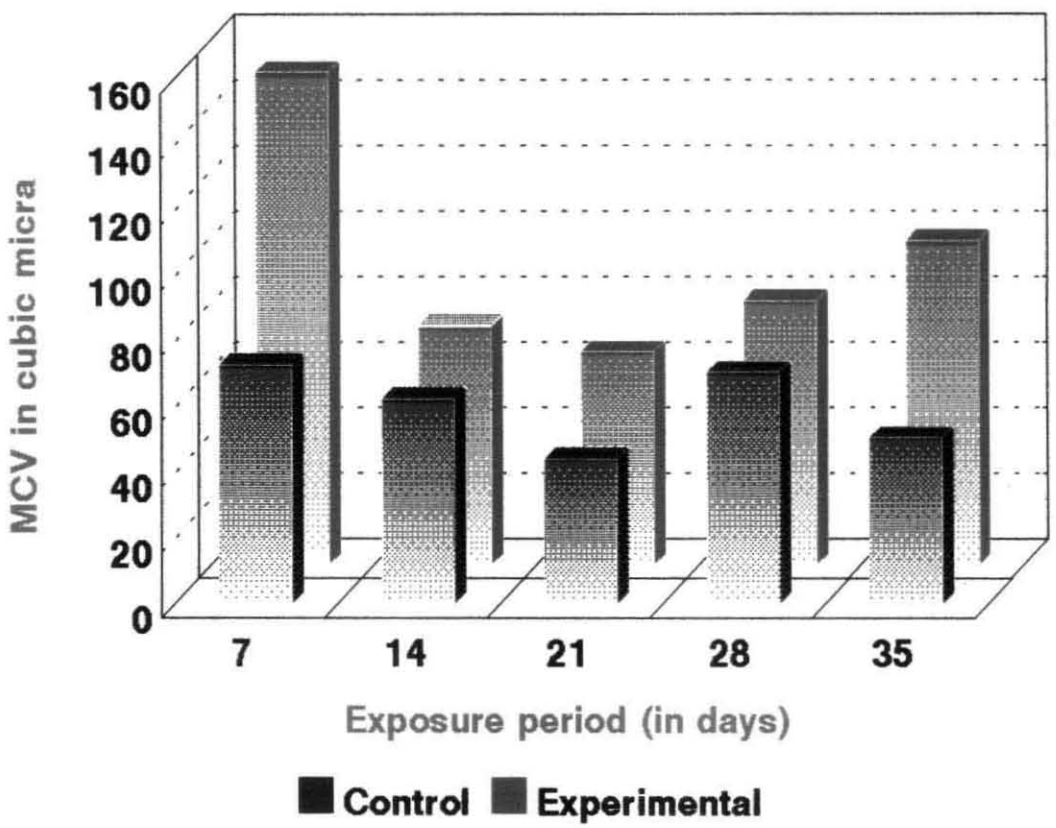
Table 29 : MCV value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| | Mean Cellular Volume value in cubic micra | | |
|---|---|---|---|
| Exposure period (in days) | Control | Treatment | Per cent change |
| 7 | 73.092 ± 3.960 | 150.222 ± 9.082 | +105.53 |
| 14 | 62.650 ± 4.778 | 72.730 ± 10.023 | +16.09 |
| 21 | 44.276 ± 4.619 | 64.980 ± 4.639 | +46.76 |
| 28 | 70.698 ± 2.273 | 80.658 ± 4.564 | +14.09 |
| 35 | 50.734 ± 2.798 | 98.780 ± 6.147 | +94.71 |

Values are mean ± S. E. of five individual observations

'+' Denotes per cent increase over control

Fig. 17. MCV value of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.




MCV value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods

Table 30 : DMRT table for Mean Cell Volume (MCV) of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $73.092^{Ba}$ | $150.222^{Db}$ | $111.657^{III}$ |
| 14 | $62.650^{Ba}$ | $72.730^{Ab}$ | $67.690^{I, II}$ |
| 21 | $44.276^{Aa}$ | $64.980^{Ab}$ | $54.627^{I}$ |
| 28 | $70.698^{Ba}$ | $80.658^{Bb}$ | $75.678^{II}$ |
| 35 | $50.734^{Aa}$ | $98.780^{Cb}$ | $74.757^{II}$ |
| Mean | $60.290^{\alpha}$ | $93.474^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 16.856 %

Analysis of period means irrespective of the treatments showed that 14th and 21st day, 14th and 28th and 35th day were on par with each other, but significantly varied from the rest of the combinations.

When treatment means were analysed irrespective of the exposure periods, the treatment groups varied significantly from that of the control group thus revealing that aluminium sulphate was toxic at sublethal level to fish *Cyprinus carpio* var. ***communis***.

# DISCUSSION

Several metals inflict anemia in fishes (Larsson, 1975; Johansson - Sjobeck and Larsson, 1978; Gill and Pant, 1987; Ruparelia *et al.*, 1990; Gill *et al.*, 1991). Haux and Larsson (1984), Tewari *et al.* (1987) and Gill *et al.* (1991) reported that anemia accompanied with reduction in hematocrit, hemoglobin and red blood cell is the most prominent hematological effect of metal intoxication in fishes.

Anemia may be due to an increase of plasma volume caused by a disturbed water balance, decreased rate of RBC production, loss or destruction of red blood cells, as suggested by Larsson *et al.* (1976). Other mechanisms concerned with anemia may include influence of metal on the life span of erythrocytes (Berlin and Friberg, 1960) or on the iron metabolism (Freeland and Cousins, 1973).

Buckley (1977) and Narain and Srivastava (1989) suggested that the low hemoglobin levels in stressed ***Oncorhynchus kisutch*** and ***Heteropneustes fossilis*** would be an expected consequences of red blood cell loss. The authors further added that inadequate hemoglobinisation or shrinkage of red blood cells can also be a cause for decreased hemoglobin and hematocrit values.

Pamila *et al.* (1991) in ***Sarotherodon mossambicus*** and Ghazaly (1992) in ***Tilapia zillii*** reported the significant lower content of hemoglobin in cadmium treated fish than control values suggesting the toxic action of cadmium on hemometabolism. Dalwani *et al.* (1985) have described the interaction between metals and enzymes involved in hemometabolism.

Erythropenia and an accompanying reduction in hemoglobin and hematocrit levels were the manifestation in fish, ***Barbus conchonius***, observed by Gill and Pant (1987) under chromium toxicosis. The authors stated that morphological aberrations in erythrocyte caused by persistent exposure to chromium resulted in reduced oxyphoretic capacity of RBC and consequently lead to tissue hypoxia; although erythropoiesis is stimulated during hypoxia (Grant and Root, 1952), due to prolonged exposure of chromium, erythropoiesis fails to keep pace with the physiological demand. Further the authors explained that organal damage caused by chromium in this fish, including capillary congestion of the secondary lamellae and telangiectasis and renal lesions may contribute significantly to secondary anemia.

In agreement to this view, Gill ***et al.*** (1991) also opined that erythroclasia (RBC destruction) surpasses the red blood cell production and release and the persistence of toxic cations override any compensatory effect of the exposed fish. Metal caused hemolysis and inhibition of erythropoiesis was reported by several authors (Albahary, 1972; Nuwde and Pearson, 1972; Houston and Keen 1984; Sakthivel, 1988).

In the present study also, aluminium exposed fishes exhibited anemic condition by recording lower content of hemoglobin, hematocrit and red blood cells than ~~that of~~ the control fish ~~values~~. This may be due to destruction of RBC or inhibition of erythropoiesis or interference of the hemometabolism or disturbance in fluid volume balance produced by aluminium ions which may find support from the above workers.

The decrease of hemoglobin leads to hypochromic microcytic anemia, which is attributed to deficiency of iron and its decreased utilization for hemoglobin synthesis; It is well known that glycolysis is concerned with the reduction of methemoglobin as soon as it is formed, thus maintaining the iron of the hemoglobin in the ferrous form, in which state only, it acts as an efficient $O_2$ carrier (Bhai ***et al.***, 1977). Koundinya and Ramamoorthy (1977) are of the opinion that the disruption of iron synthesizing machinery due to inhibition of aerobic glycolysis could be the reason for the decrease of blood parameters in the stressed fish. In the present study, a similar type of mechanism may be operating resulting in the reduction of hemoglobin count in ***Cyprinus carpio*** var. ***communis***.

Gill and Pant (1987) recorded in ***Puntius conchonius*** after mercury exposure, an initial fall in RBC and hemoglobin count, but after 2 or 3 weeks, increment occurred in both these indices. The authors described that incipient decrease could be due to hemolysis caused by mercury, while subsequent recovery could be ascribed to enhanced erythropoiesis which is triggered as typical stress response. In agreement to this concept, Witters ***et al.*** (1990) reported that rising catecholamine concentrations stimulated spleenic contraction to increase the number of circulating erythrocytes.

Houston (1973), Lane (1979) and Saad ***et al.*** (1973) reported that in fishes, blood constituents such as hemoglobin, RBC and others have rapidly increased under the stress of hypoxia. In our present sublethal studies, the occurrence of gradual

recovery in RBC and hemoglobin may be related to enhanced erythropoiesis as a stress response or hypoxia induced recovery as reported by the above authors.

Folmar (1993) stated that increases in hematocrit values were associated with osmotic shifts; as the pH of the blood decreased, erythrocytes swelled and plasma volume decreased. The findings of McKim ***et al.*** (1970), Vander Putte ***et al.*** (1982) and Hilmy ***et al.*** (1987) support the above view, that RBC swelling is the reason for hematocrit increase in fishes after exposure to chromium, copper, mercury and zinc, respectively.

The above concept is further strengthened by the observation of higher value of hematocrit in fishes by aluminium exposure in acidified waters (Goss and Wood, 1988; Wood ***et al.***, 1988b; Dietrich and Schlatter, 1989a; Playle ***et al.***, 1989; Witters ***et al.***, 1986, 1990; Reid ***et al.***, 1991). Higher values of hematocrit observed in the present study after 7th and 35th week may find support of the above author's explanation that the RBC swelling consequent to fluid volume disturbance might have increased the hematocrit value.

Leucocytosis observed by the toxic effect of manganese in ***Heteropneustes fossilis*** is an adaptation to meet the stress condition in fish as reported by Garg ***et al.*** (1989). Saad ***et al.*** (1973) stated that oxygen deficiency is responsible for abundance of leucocytes. McLeay and Brown (1974) and Singh (1995) explained that leucocytosis is probably due to the tissue damage and subsequent removal of cell debris by Cu and Cr exposure in ***Channa punctatus***. Haniffa (1989) and Nelson and Rani (1992) have also supported this view by the fact that toxicant damages gill

tissues and function, leading to leucocytosis. Lymphocytosis was observed by Chitra and Ramana Rao (1986) in ***Channa punctatus*** by mercury intoxication, which stimulates lymphopoiesis as a direct stimulation of the immunological mechanism against stressor present in the media.

In line with above author's results, in the present study also, leucocytosis was observed in both acute and sublethal exposures of aluminium to meet stress conditions which might have produced hypoxia and gill damage in fishes.

Ruparelia ***et al.*** (1991) suggested that red blood cell swelling is the cause for the decrease in MCHC value in ***Oreochromis mossambicus*** after cadmium exposure. Nikinmaa (1982) and Vuorinen ***et al.*** (1992) reported that lowered MCHC, elevated hematocrit denotes the swelling of erythrocytes, a mechanism by which blood $O_2$ transport capacity is increased when fishes were subjected to less effective gas exchange (Vuorinen ***et al.***, 1992). Besides, lowered MCHC might result from the release of young erythrocytes containing less hemoglobin than the older ones (Lehmann and Sturenberg, 1975) into the circulation (Vuorinen ***et al.***, 1990).

In support of the above concept many investigators recorded decline in MCHC value ***viz.***, by exposing aluminium to fish in acidified water (Goss and Wood, 1988; Wood ***et al.***, 1988a; Vuorinen ***et al.***, 1992). In the present study the decrease in MCHC value may be due to red blood cell swelling or release of young erythrocytes containing less hemoglobin into the circulation.

Ghazaly (1992) reported reduction in MCH value in ***Tilapia zillii*** after cadmium poisoning. Further, the author stated that these hematological changes in fish

compensate for impaired oxygen uptake due to gill damage caused by cadmium. In the present study, similar hematological changes might have occurred due to aluminium exposure.

The increased mean cell volume (MCV) during stress (capture) was due to the swelling of red blood cells or to the release of large red blood cells into the general circulation as reported by Fletcher (1975). The anaerobic condition (hypoxia) probably developed during sublethal aluminium stress (Poleo, 1995) may be the reason for increased mean cell volume as indicated by Soivio and Nyholm (1973) that anaerobic storage of trout red blood cells caused them to swell.

# BIOCHEMICAL COMPONENTS

# INTRODUCTION

Cannon (1929) reported that all organisms maintain their 'internal milieu' more or less constant by variety of regulatory mechanisms. When the level of toxicants in environment exceeds the limits of the assimilatory capacity of these regulatory mechanisms, it leads to biochemical changes and finally results in death (Aruna and Gopal, 1987). Hence early detection of specific physiological abnormalities provide an indication of exposure, prior to manifestation of any gross damage (Palmes, 1976). Gill and Pant (1981) reported that the measurement of biochemical changes in blood and tissues of fish under exposure to the toxicant may be used to predict effects upon chronic exposure.

Alterations in the physiological and biochemical parameters of toxicant treated fish have recently emerged as an important tool for the water quality assessment and to know the pathological status of fish in the field of environmental toxicology (Racicot ***et al.***, 1975; Wieser and Hinterleintner, 1980). Mayer ***et al.*** (1992) suggested that the biochemical changes occurring in the body give early indication of stress.

Blaxhall (1972) reported that fish live in intimate contact with their environment and therefore are very susceptible to physical and chemical changes which may be reflected in their blood components. Bouck (1951) stated that environmental stress causes a variety of detectable and recognisable changes in the blood of fish. Significant alterations in tissue metabolism could be predicted based

upon changes in blood components (Cornish and Moon, 1986) which have been suggested as an indication of stress in fishes (McLeay, 1971; Wedemeyer, 1972).

Heavy metals constitute the most widely distributed group of highly toxic and long retained substances; almost all metals are toxic in higher concentrations and some are highly poisonous even at low level; due to heavy metal complexation with biological molecules, normal functioning of the cell is disturbed and this in turn may result in variation in the fundamental, biochemical and physiological mechanisms of animals (Rothestein, 1970; Fowler, 1972).

According to Pagenkopf (1983), a biological response from a target or to accumulate within the organism, a metal must interact/traverse a cell membrane. Binding at the cell surface site might precede transport into the cell and reacts with the metabolically active centre within the cell (Harrison and Morel, 1983; Sunda and Huntsman, 1983).

Exley ***et al***. (1991) proposed that aluminium binds with cell membranes and increases both membrane rigidity and permeability more effectively; this binding is likely to induce membrane changes leading to increased aluminium entry into cell where it can exert secondary toxic effects. Aluminium ions also might act as cross linkers forming connecting bridges between proteins or other molecules (Ganrot, 1986; Exley ***et al***., 1991).

Alexander and Ingram (1980) reported that the concentration of protein in the serum of fish has been used as an indicator of their general state of health. Proteins exhibit functional versatility and are therefore, involved in a variety of

biological roles such as transport, metabolic control, contraction and catalysis of chemical transformation (Christian *et al.*, 1993). Measurement of total protein provides an insight on the biochemical mechanism of metabolism under stressful conditions (Keitly and Stehly, 1989). Umminger (1970) reported that proteins are the energy source ~~to be spared~~ during chronic period of stress.

The toxicological and physiological responses of juvenile and adult fish to aluminium exposures are varied and complex and they depend primarily on the pH and speciation of the ambient water (Reader *et al.*, 1989). Increased plasma protein level was observed in rainbow trout, ***Oncorhynchus mykiss***, (Goss and Wood, 1988), in ***Salvelinus fontinalis*** (Wood *et al.*, 1988a) and in white fish, ***Coregonus wartmanni***, (Vuorinen *et al.*, 1990) exposed to aluminium at low pH. In support of this, Witters *et al.* (1990a), McDonald *et al.* (1991) and Reid *et al.* (1991) found elevated plasma protein content in fish when exposed to aluminium in acidic pH.

Wood *et al.* (1988a) and Vuorinen and Vuorinen (1991) noted no change in plasma protein at pH 4.5 + 333 μg Al and at pH 5.0 and 6.0 with aluminium in fish ***Salvelinus fontinalis*** and white fish, ***Coregonus wartmanni***, respectively. Waring and Brown (1995) also found no changes in plasma protein concentrations while exposing brown trout, ***Salmo trutta***, to pH 5.0+ Al.

Plasma glucose has been widely monitored to study stress in fish (Mazeaud *et al.*, 1977; Donaldson, 1981; Pickering *et al.*, 1982). Silbergeld (1974) stated that assay of this important blood parameter can serve as an indicator of environmental stress. The increase in blood glucose of fish is an usual response to

most stressors (Wedemeyer, 1972; Soivio and Oikari, 1976; Watson and McKeown, 1976; Casillas and Smith, 1977; Saad *et al.*, 1973; Zelnik and Goldspink, 1981; Vijayan and Moon, 1992; Ramesh, 1995; Waring and Brown, 1997). Elevation in blood glucose level has been used by Wedemeyer (1972) and Nemcsok and Boross (1982) for demonstrating metabolic stress. The elevation in blood glucose and the resultant hyperglycemic responses have been attributed to the release of hyperglycemic hormones and subsequent mobilisation of glycogen reserves as suggested by Mayer *et al.* (1992).

Acute heavy metal exposures cause increased concentration of glucose in plasma of fish (Watson and McKeown, 1976; Shaffi, 1980; Chaudhry, 1984; Sivakumari, 1998). However, chronic exposure of fish to heavy metals has been reported to either increase (Sastry and Subhadra, 1985) or decrease plasma glucose levels (Christensen *et al.*, 1977; Gill and pant, 1981; Sastry and Sunitha, 1983; Haux and Larsson, 1984; Bleau *et al.*, 1996).

Heavy metal cadmium induced hyperglycemia in rainbow trout ***Oncorhynchus mykiss*** (Haux and Larsson, 1984), in freshwater teleost ***Notemigonus chrysoleuca*** (Benson *et al.*, 1987) and in ***Salvelinus fontinalis*** (Christensen *et al.*, 1977). Other heavy metals *viz.*, copper (Christensen *et al.*, 1972; Nemcsok and Hughes, 1988; Singh and Reddy, 1990), lead (Tewari *et al.*, 1987) chromium (Strik *et al.*, 1975) , mercury (Bleau *et al.*, 1996) and zinc (Watson and McKeown, 1976; McLeay, 1977) enhanced blood glucose levels in various species of fish. Hypoglycemia was also recorded in ***Salvelinus fontinalis*** exposed to cadmium

(Christensen *et al.*, 1977) and in ***Salmo gairdneri*** treated with lead (Haux and Larsson, 1972).

Exposure of rainbow trout, ***Salmo gairdneri*** (Goss and Wood, 1988; Playle *et al.*, 1989), brown trout, ***Salmo trutta*** (Whitehead and Brown, 1989; Waring and Brown, 1997) and whitefish, ***Coregonus wartmanni*** (Vuorinen and Vuorinen, 1991; Vuorinen *et al.*, 1990, 1992) to aluminium in acidic water increased plasma glucose level.

The foregoing review of literature reveals that not much work has been done on the impact of aluminium toxicity on biochemical parameters of fish at neutral pH. Hence, the present investigation is aimed to study the magnitude of secondary stress responses (plasma protein and glucose) in ***Cyprinus carpio*** var. ***communis*** exposed to aluminium toxicity in neutral pH.

# MATERIAL AND METHODS

Plasma samples from fish treated with aluminium sulphate at acute and chronic levels were taken as per the procedure outlined in Chapter -III for protein and glucose estimation.

## PROTEIN

Total protein concentration was analysed following Biuret method as described by Strickland ***et al.*** (1961) and Henry and Winkelman (1974) using Autopak protein diagnostic reagent kit manufactured and supplied by Miles India Ltd., Baroda, India (Code 6370) by Technicon RA-500 system (Technicon Instruments Corporation, Tarrytown, New York, USA).

### Principle

Peptide bonds of protein form a blue violet colored complex with cupric ions in an alkaline medium. The intensity of the colour is proportional to the number of peptide bonds and the color is read at 540 nm (530-570nm). The final color is stable for 8 hours.

### Reagents

| | | |
|---|---|---|
| Reagent 1 | : | Biuret reagent |
| Reagent 1A | : | Surfactant reagent |
| Reagent 2 | : | Standard albumin reagent (7 g/dL) |

**Solution A**

**Working solution**

To the whole volume of biuret reagent (Reagent 1), 81.00 ml of distilled water was added. Then whole volume of surfactant reagent (Reagent 1A) was added and mixed gently to avoid foaming.

**Procedure**

The Technicon RA-500 system, a computer controlled, random access clinical chemistry analyser system was setup for the analysis of total plasma protein concentration in the sample. Before starting the experiments, the calibration factor was found out. This value was determined ***via***., a calibrator assay according to the instruction in the chemistry program data sheet for ***in vitro*** measurement of total protein in the Technicon method manual of Technicon RA-500 system. Once if the calibration and program setting was done the instrument functions according to the fed values until the next calibration.

After setting the instrument, the working solution (Solution A) was placed in the reagent tray and the sample from control and experimental groups were placed in appropriate grooves of the sample tray. Then, 2 function was operated followed by idee number (according to our convenience so as to identify the sample) in order to download the worklist by giving a particular assay code. When the worklist has been cultured and operate command has been given, the Technicon RA-500 system operates automatically and 375 µl of solution A for each test and 7.5 ml of sample from the respective vials were added to the reaction tray containing fixed

cuvettes. The absorbance of the sample was measured and calculated as given below by the computing unit and the total protein concentration in g/dl was reported as printed matter within 2 min.

**Calculation**

Total protein concentration in g/dl was calculated as follows.

$$\text{Reported result in g/dl} = \frac{\text{Assay result x Unit factor - Intercept}}{\text{Slope}}$$

Where,

Assay result = Calibration Factor x Assay Absorbance.

**GLUCOSE**

True enzymatic glucose was estimated according to the method of Trinder (1969), Bergmayer (1974) and Young ***et al.*** (1975) enzymatically by glucose oxidase/peroxidase method by Autozyme glucose diagnostic reagent kit manufactured by Accurex Biomedical, Bombay India (Code GU-3s) using Technicon RA-500 (Technicon Instruments Corporation, Tarrytown, New York).

**Principle**

Glucose is converted to gluconic acid by Glucose oxidase (GOD). Hydrogenperoxide formed in this reaction, in presence of peroxidase (POD), oxidatively couples with 4-amino antipyrine/phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm (500-550 nm). The intensity of the color complex is directly proportional to the glucose in specimen.

The reaction can be represented as follows.

$$\text{ß-D Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4 amino antipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Red Dye} + H_2O$$

**Reagents**

Reagent 1 : Enzyme vial

Reagent 2 : Diluent reagent

Reagent 3 : Standard glucose reagent (100 mg/dL)

**Solution A**

**Working Solution**

To the working solution bottle the content of enzyme vials were transferred. Then the diluent reagent was added and mixed gently by swirling or inversion. Care was taken to avoid shaking vigorously.

**Components and concentration of working solution**

| | | |
|---|---|---|
| Phosphate buffer, pH 7.0 | = | 170.00 mMol/L |
| Glucose oxidase | = | 5000.00 IU/L |
| Peroxidase | = | 3000.00 IU/L |
| 4 amino antipyrine | = | 0.28 mmol/L |
| Phenol | = | 16.00 mMol/L |

Stabilizers and inactive ingredients

**Procedure**

The Technicon RA-500 system, is a computer controlled, random access clinical chemistry analyser system. It was setup for the analysis of true enzymatic

glucose in the plasma. For this, first the calibration factor was determined. This value was determined ***via***, a calibrator assay according to the instruction in the chemistry program data sheet for ***in vitro*** measurement of enzymatic glucose in the Technicon method manual of Technicon RA-500 System. The calibration and program setting is done for one time before running the experiments.

The plasma from control and experimental groups were placed in appropriate grooves of the sample tray. Then the 2 function and ~~idee~~ i.d. number (given according to our convenience so as to identify the sample) were operated to enter worklist and download the worklist by giving a particular assay code. Now the Technicon RA-500 System operates automatically and 350 µl of solution A for each sample and 7.5 µl of plasma from the respective vials were added separately to the respective fixed cuvettes in the reaction tray. The contents were incubated for 30 sec. and the absorbance of the samples ~~were~~ was measured individually. The computing unit calculated the true enzymatic glucose level in mg/dl as given below, and printed out within 1 min.

**Calculation**

True enzymatic glucose in mg/dl was calculated as follows.

$$\text{Reported result in mg/dl} = \frac{\text{Assay result x Unit factor - Intercept}}{\text{Slope}}$$

Where,

Assay result = Calibration Factors x Assay Absorbance.

# RESULTS

Acute concentration of aluminium sulphate caused significant decrease in protein level in the tested fish than that of its control showing a per cent decline of 21.74 whereas glucose level increased significantly in the experimental fish exhibiting a per cent increase of 51.79% (Table 31, Fig. 18) at the end of 24 h experiment. Alterations in both the parameters were significant at 5 per cent level.

Fish treated with sublethal concentration of aluminium sulphate showed a reduction of 28.13 per cent in the plasma protein level than that of the control after 7th day (Table 32, Fig. 19). However, after 7th day, an increase in plasma protein level was noticed recording 7.14 and 1.05 per cent at the end of 14th and 21st day, respectively. In the subsequent weeks, plasma protein showed a substantial decrease in their levels in treated fish compared to that of the control registering 33.33 and 46.82 per cent decline after 28th and 35th day, respectively.

Testing the changes in plasma protein by DMRT (Table 33) showed that both in the control and experimental groups, its level was on par with each other at the end of 7th day. A similar result was also observed in the rest of the exposure periods. Analysis of protein levels from the control groups for the varying periods showed that 7th, 21st and 28th day and 14th, 28th and 35th days were on par with each other. But they varied significantly from that of the other combinations. Similarly analysis of different treatment periods revealed that 7th, 28th and 35th days, 14th and 21st day

Table 31 : Protein and Glucose levels in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminum sulphate toxicity.

| S .No | Parameters | Control | Experimental | Per cent change | Calculated ' t' |
|---|---|---|---|---|---|
| 1 . | Protein (g/dl) | 2.300 ± 0.182 | 1.800 ± 0.0316 | - 21.74 | 2.7116 * |
| 2 . | Glucose (mg/dl) | 112.000 ± 1.643 | 170.000 ± 1.342 | + 51.79 | 27.3415* |

Values are mean ± S.E.of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Values are significant at 5% level

Degrees of freedom at 8 t 0.05 = 2.306

Fig. 18. Protein and glucose levels in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.

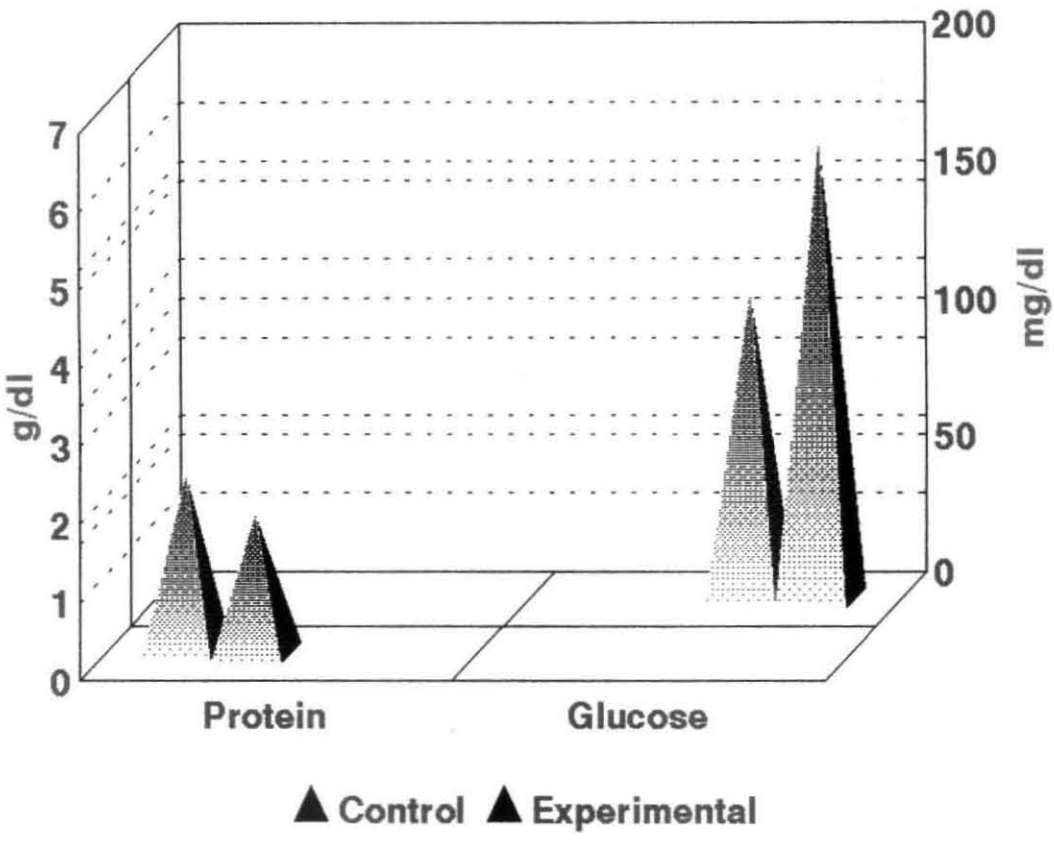

200
150
100
50
0
mg/dl
7
6
5
4
3
2
1
0
g/dl
Protein
Glucose
Control
Experimental

Fig. 18

Table 32 : Protein level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium sulphate toxicity for varying periods.

| Exposure period in days | Plasma protein level in g/dl | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 3.200±0.207 | 2.300+0.501 | -28.13 |
| 14 | 4.200±0.187 | 4.500±0.367 | +7.14 |
| 21 | 3.800±0.266 | 3.840±0.317 | +1.05 |
| 28 | 3.600±0.472 | 2.500±0.279 | -33.33 |
| 35 | 4.400±0.766 | 2.340±0.354 | -46.82 |

Values are mean ± S.E.of five individual observations

'-' Denotes per cent decrease over control

'+' Denotes per cent increase over control

Fig. 19. Protein level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

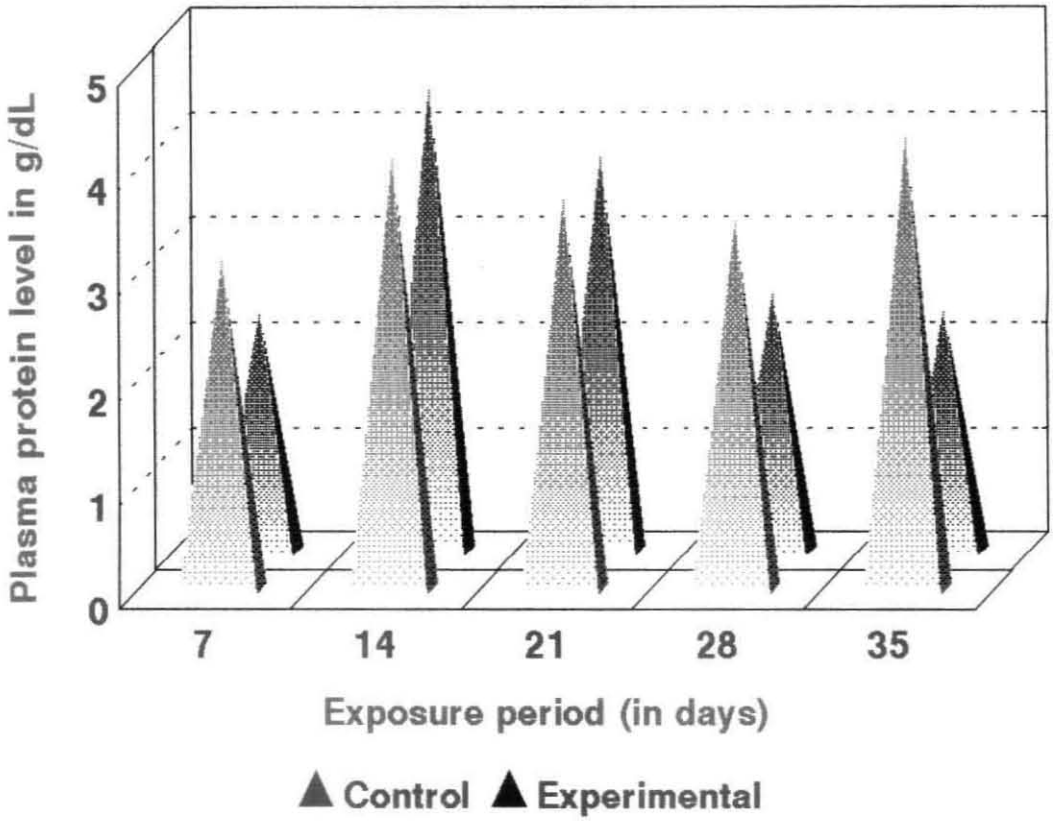

Plasma protein level in g/dL
5
4
3
2
1
0
7
14
21
28
35
Exposure period (in days)
Control
Experimental

Fig. 19

Table 33 : DMRT table for protein level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $3.200^{Aa}$ | $2.300^{Aa}$ | $2.750^{I}$ |
| 14 | $4.200^{Ba}$ | $4.500^{Ba}$ | $4.350^{III}$ |
| 21 | $3.800^{Aa}$ | $3.840^{Ba}$ | $3.820^{II, III}$ |
| 28 | $3.600^{ABa}$ | $2.500^{Aa}$ | $3.050^{I, II}$ |
| 35 | $4.400^{Ba}$ | $2.340^{Aa}$ | $3.370^{I, II}$ |
| Mean | $3.840^{\alpha}$ | $3.096^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Roman letters represent comparison of period means
Greek letters represent comparison of treatment means
Degree of freedom at F <0.05
CV = 26.184 %

were on par on with each other, but varied significantly from the rest of the combinations.

Analysis of period means irrespective of the treatment showed that 7th, 28th and 35th days, 21st, 28th and 35th days and 14th and 21st days were on par with each other, while they varied significantly from the rest of the combination. Analysis of treatment means irrespective of the exposure period showed that the treatment mean varied significantly from that of the control. The above analysis reveals that protein level was significantly affected during sublethal aluminium stress.

Table 34 and Fig. 20 give the data on the changes in plasma glucose levels of fish from sublethal studies. A marked rise in the plasma glucose level was observed in the treated fish than that of the control fish throughout the experimental period and it was directly proportional to the duration of exposure. An initial increase of 39.22 per cent was observed after 7th day, which shot up to 113.28 per cent after 35th day.

Table 35 gives the data on analysis of plasma glucose level by DMRT, during sublethal aluminium sulphate stress. At the end of the 7th day plasma glucose level varied significantly ~~than that~~ of the control. A similar trend was also observed during the rest of the experimental period.

When control group alone was tested for varying periods 14th, 28th day and 7th, 14th 21st and 28th day were on par with each other, whereas the rest of the exposure periods were significantly different from those groups and among themselves. Similarly when treatment alone was analysed for varying exposure

Table 34 : Glucose level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Plasma glucose level in mg/dl | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 127.400±7.347 | 177.500±1.830 | +39.22 |
| 14 | 120.000±1.639 | 180.000±1.721 | +50.00 |
| 21 | 125.800±2.219 | 203.800±1.485 | +61.90 |
| 28 | 114.000±1.579 | 232.500±1.899 | +103.95 |
| 35 | 128.000±1.414 | 273.000±1.154 | +113.28 |

Values are mean ± S.E.of five individual observations

'+' Denotes per cent increase over control

Fig. 20. Glucose level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

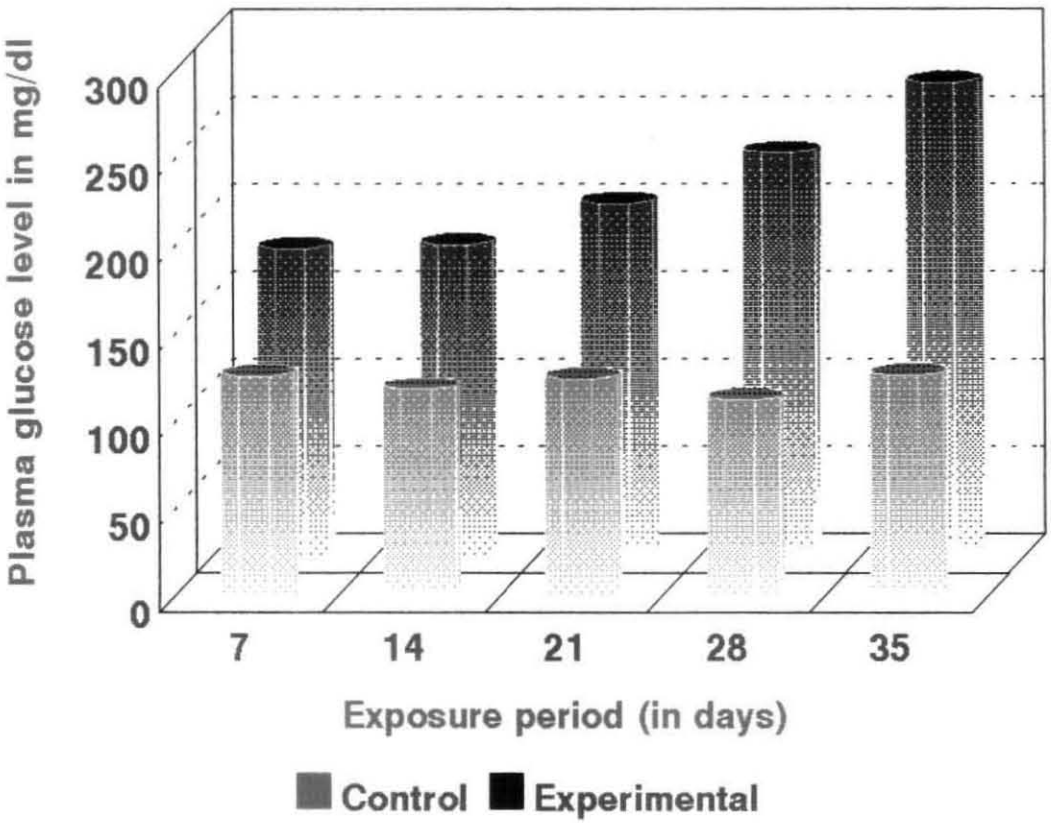

Plasma glucose level in mg/dl
300
250
200
150
100
50
0
7
14
21
28
35
Exposure period (in days)
Control
Experimental

Fig. 20

Table 35 : DMRT table for glucose level in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentrations of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $127.400^{Ba}$ | $177.500^{Ab}$ | $152.400^{I}$ |
| 14 | $120.000^{ABa}$ | $180.000^{Ab}$ | $150.00^{I}$ |
| 21 | $125.800^{Ba}$ | $203.800^{Bb}$ | $164.800^{II}$ |
| 28 | $114.000^{Aa}$ | $232.500^{Cb}$ | $173.200^{III}$ |
| 35 | $128.000^{Ba}$ | $273.000^{Db}$ | $200.500^{IV}$ |
| Mean | $123.040^{\alpha}$ | $213.320^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Roman letter represent comparison of period means
Greek letters represent comparison of treatment means
Degree of freedom at F <0.05
CV = 3.755 %

periods, only 7th and 14th day were on par with each other, but they varied significantly from that of 21st, 28th and 35th days, which also significantly varied among themselves.

Analysis of period means, irrespective of the treatment also exhibited a similar trend like that of the treatment group. When treatment means were analysed irrespective of their exposure periods, the treatment group varied significantly ~~than that of~~ the control group thus indicating that aluminium exposure is toxic to fish at sublethal levels too.

# DISCUSSION

Vallee and Ulmer (1972) reported that metals show a strong affinity for ligands such as phosphates, cystenyl and histidyl side chain of proteins, purines and porphyrins, and are capable of forming complexes with ligands containing sulphur, nitrogen and oxygen as electron donors.

Goodman and Gillman (1970) observed that most toxicants are transported reversibly bound to blood protein with only small portion existing in the free or unbound state. According to Petermann (1961), toxicants must usually exist in the free state to react with biological molecules and interfere with biochemical mechanisms. The author also emphasized the need of the plasma protein availability for the binding and transporting of toxic substances which may play an important role in the toxicological consequences of these compounds.

Klaassen (1980) is of the opinion that several plasma proteins mostly albumin within plasma bind normal physiological constituents in the body as well as some foreign compounds. The author further stated that the fraction of toxicant in the plasma bound to the plasma protein is not immediately available for distribution into the extravascular space or for filtration at the kidney.

Whole body protein concentrations are influenced by a variety of environmental factors (Claybrook, 1983). Under conditions of stress many organisms will mobilize proteins as an energy source (Florkin and Scheer, 1970; Gilles, 1970; Bayne, 1973a, b). Rapid utilization of protein by the fishes under pollutant stress has

been reported by Webb and Brett (1973), Vasanthi *et al.* (1990), and Palanichamy and Baskaran (1995).

Srivastava and Srivastava (1984) are of the opinion that under stress, fish may resort to alter native metabolic pathways mediated by a reduction in the activity of Nissl and Golgi granules. Neff (1985) stated that decline in protein content may be related to impaired food intake, increased energy cost for homeostatis, and tissue repair and detoxification mechanisms during stress. Reduction in protein synthesis has also been reported to result from the inhibition of formation of new peptide chains and the dissociation of 80S ribosome as propounded by Colombo *et al.* (1958), Jenkins *et al.* (1970) and Rao *et al.* (1987).

Chandravathy and Reddy (1991) stated that decrease in protein might imply degradation of protein into aminoacids causing an elevation of aminoacid pool. The aminoacids further could be used for gluconeogenetic purposes. Such reports have been documented by several authors in fishes under toxicant stress (Malla Reddy and Bashamohideen, 1988; Baskaran *et al.*, 1989b; Chandravathy and Reddy, 1991). Diminished incorporation of aminoacids into the protein also might lead to decrease in protein content as opined by Dhar and Banerjee (1983).

The depletion of protein content may suggest increased proteolysis and possible utilization of the products of their degradation for metabolic purposes. The products which may be fed into TCA cycle through amino transferase system to ~~cope up with~~ satisfy the excess demand of energy during toxic stress conditions as observed in freshwater fish ***Anabas scandens*** after exposure to lead nitrate (Chandravathy and Reddy, 1994).

Increased adrenocorticosteroid output by nickel exposure in ***Cyprinus carpio*** (Thatheyus ***et al.***, 1992) was linked ~~for~~ with protein depletion. Ghosh and Chatterjee (1989) suggested that protein depletion by toxicant exposure is probably due to (i) direct utilization by cells for energy requirements (ii) formation of lipoproteins which are utilized for repairing of damaged cells and tissue organelles and (iii) increased lipolysis. The decrease in protein content in the present study during acute and sublethal treatments with aluminium may be due to rapid utilization of protein by fishes, ~~or~~ increased proteolysis or reduced protein synthesis under aluminium stress recalling the observations of the above authors.

However, in the present study after second and third week of sublethal exposure to aluminium, plasma protein content of ***Cyprinus carpio*** var. ***communis*** showed recovery. This may be due to an increase in the rate of protein synthesis as a 'General Adaptation Syndrome' to bind the excess aluminium ion in the plasma, as aluminium has a very high affinity for a large number of proteins, polynucleotides and glycosamine glycans and possibly binds with phosphorylated proteins and phosphorylated histones (Ganrot, 1986; Martin, 1994). This view finds support from the report of Fry (1971) and Ramalingam and Ramalingam (1982).

Bouck and Ball (1965) also demonstrated that low $O_2$ concentrations altered the protein composition of serum in blue gill, ***Lepomis macrochirus***, and largemouth bass, ***Micropterus salmoides***. Bouck (1972) found in rockbass, ***Ambloplites repestris*** undergoing sublethal hypoxia, a significant increase in plasma protein, which was attributed to cellular enzymes entering the blood. Hypoxia,

normally observed after aluminium exposure in fishes (Poleo, 1995) might have also contributed for the increase in plasma protein content in the present study.

Rosseland and Staurnes (1994) described that main consequences contributing to the death of fish during acute aluminium exposure concentration are respiratory, ionoregulatory and circulatory disturbances. In addition to the above characteristic symptoms, increased blood protein concentration also leads to increased blood viscosity. Consequent to ion disturbance, the protein and amino acids participate actively since they are osmotic effectors as well as components in branchial buffering systems (Harper, 1979; Jurss, 1980). In the present study during sublethal treatment the elevation in protein content might act as an osmoeffector particle consequent to occurrence of ion disturbances produced by aluminium stress in ***Cyprinus carpio*** var. ***communis***.

An elevated blood glucose level is an usual response under most stressors (Sibergeld, 1974; Donaldson and Dye, 1975; Casillas and Smith, 1977; Wedemeyer and McLeay, 1981; Scherer ***et al.***, 1986; Bleau ***et al.***, 1996; Waring and Brown , 1997). Xenobiotics of diverse origin produced hyperglycemia and hepatic glycogen depletion in various teleost species (McLeay, 1977; Koundinya and Ramamurthi, 1977; Ramesh ***et al.***, 1995; Sivakumari,1998).

Bhattacharya ***et al.*** (1987) recorded hyperglycemia with a concomitant depletion of hepatic glycogen to short term exposure of cadmium, mercury and the mixture of industrial pollutants, whereas to long term exposure hypoglycemia was observed with persistent depletion of hepatic glycogen content in ***Channa punctatus***.

The authors suggested that hepatic glycogen is the source of hyperglycemia and maximal glycogen depletion corresponded to a dramatic increase in blood glucose levels, indicating some of the hepatic glycogen is converted into glucose.

Stresses elicit physiological disturbances in fish and mediate ***via*** the hypothalmo pituitary interrenal axis, involving the hormones corticotropin and cortisol and through the catecholamines from the chromaffin tissues (Schreck, 1981). Wedemeyer (1969) found that circulatory glucocorticoids and catecholamines are secreted in increased amounts after stress in teleostean species; both groups of these adrenal hormones, especially catecholamines in very low doses, produce hyperglycemic effects in fish. Glucose is released by corticosteroids whose elevation has been described as a primary response to most stressors including metals (Vuorinen and Vuorinen, 1991; Ghazaly, 1992; Varghese ***et al.***, 1992; Bleau ***et al.***, 1996).

Pickering (1993) reported that the hypothalamo-pituitary interrenal axis, activated by numerous stressors, elevated blood levels of cortisol . This endocrine response is preceded by a surge of catecholamines which stimulates lipolysis, glycogenolysis and gluconeogenesis which are important energy mobilizing processes (Vijayan and Moon, 1992).

From the review of Folmar (1993) it is evident that ~~there is~~ a close association exists between the increase in serum cortisol and glucose level. The authors have further reported that the rapid rise in glucose results from glycogenolysis (release of glycogen reserves in muscle and liver) initiated by catecholamines while sustained elevation of serum glucose ~~are~~ is maintained by cortisol stimulated

gluconeogenesis (protein metabolism). A similar rise in glucose level concurrent with elevation of plasma cortisol was observed after aluminium exposure to various species of fishes (Goss and Wood, 1988; Wood ***et al.***, 1988b; Whitehead and Brown, 1989; Waring and Brown, 1997).

Goss and Wood (1988) recorded an apparent increase in blood glucose level in rainbow trout ***Oncorhynchus mykiss*** under acute aluminium in acidic waters. The authors have recorded a simultaneous increase in plasma cortisol and greater ionoregulatory disturbance. These results supports the phenomena that plasma cortisol mediates glycogenolysis and the resultant hyperglycemia aids in regulation of plasma osmolarity. Under conditions of stress, hyperglycemia may provide additional energy during times of metabolic needs such as fight or flight response (Goss and Wood, 1988).

In the present study also, in both acute and sublethal studies, the rise in plasma glucose level might have been elevated by similar mechanism caused by aluminium stress supporting the findings of above authors. Hypoxia, is a stimuli to increased circulating glucose level as an anaerobic energy source; in hypoxic condition, the respiratory metabolism is depressed and therefore, stored intracellular glycogen is utilized; under such conditions, hyperglycemic hormone is released for the degradation of glucose; this glucose leaks into the blood causing hyperglycemia (Telford, 1974). The hypoxia usually observed after aluminium exposure might be a probable reason for the increased blood glucose level in the present study.

Hyperglycemia may be a physiological response to meet the critical need of the brain tissue for increased energy in the form of glucose during exposure to toxicants (Mazeaud ***et al.***, 1977; Donaldson, 1981; Pickering ***et al.***, 1982). But according to McDonald (1983a, b) and Brown ***et al.*** (1984) fish are not using the elevated glucose to derive extra energy alone as the increase in standard metabolic rate was not evident. Hence, the present observation supports the theory speculation that glucose may play a more passive role in biochemical adaptation to losses of $Na^+$ and $Cl^-$ to maintain osmolarity in the face of declining ion levels (McDonald, 1983a, b; Brown ***et al.***, 1984; Hegab and Hanke, 1984; Edwards ***et al.***, 1987; Vuorinen ***et al.***, 1990; Reddy and Rao, 1991).

Hyperglycemia observed in the present acute and sublethal studies may be due to cortisol induced glycogenolysis and gluconeogenesis or hypoxia causing degradation of glycogen to glucose as an anaerobic energy source as a biochemical adaptation to maintain ion-balance.

# ENZYMES

# INTRODUCTION

Pollutants in the aquatic ecosystems affect growth and enzymes in aquatic organisms (Webb and Brett, 1973). The occurrence of toxicity in metal exposed animals corresponds to an overwhelming capacity of accumulated metal binding to macro molecules especially cytosolic enzymes (Winge ***et al.***, 1974; Brown and Parsons, 1978; Pruell and Englchardt, 1980; Brown ***et al.***, 1984). Recently there has been increasing interest in using changes in enzyme activity in aquatic animals as an index reflecting metal toxicity (Sastry and Sunitha, 1983; Lan ***et al.***, 1993a, b; 1995).

$Na^+$ $K^+$ ATPase plays a central role in whole body ion regulation by providing energy for the movement of $Na^+$ $K^+$ across the cellular membranes against an electrochemical gradient which is required for the uptake of metabolites such as glucose and aminoacids by cells (Epstein ***et al.***, 1967; Trachtenberg, 1981; de Renzis and Bornancin, 1984; Evans, 1987). Regeneration of transmembrane potential during nerve excitation, transmembrane movement of $Ca^{2+}$ during muscle stimulation and maintenance of osmotic equilibrium in cells are some of the functions attributed to $Na^+$ $K^+$ ATPase (Quinn and Lane, 1966; Spencer ***et al.***, 1979; Haya and Waiwood, 1983).

The xenobiotics can alter $Na^+$ $K^+$ ATPase activity due to disruption of energy producing metabolic pathways or interact directly with the enzyme (Watson and Beamish, 1980). Gill $Na^+$ $K^+$ ATPase activity was affected in smolts of Chinook

salmon, ***Oncorhynchus tshawytscha***, by copper intoxication in natural spring water (Beckmann and Zaugg, 1988). In juvenile rainbow trout, ***Oncorhynchus mykiss***, and in flounder, ***Platichthys flesus***, Lauren and McDonald (1987) and Stagg and Shuttleworth (1982) found depression of $Na^+$ $K^+$ ATPase activity by copper respectively.

Decreases in $Na^+$ $K^+$ ATPase activity by chromium on rainbow trout ***Oncorhynchus mykiss*** (Kuhnert and Kuhnert, 1976), by Vanadium on ***Clarius batrachus*** (Shah *et al.*, 1996), Zn on ***Oncorhynchus mykiss*** (Watson and Beamish, 1981) and cadmium on freshwater teleost (Watson and Benson, 1987) and in eels, ***Anguilla anguilla***, (Lemaire-Gony and Mayer-Gostan, 1994) were also reported. Cadmium exposure to the American lobster, ***Homarus americanus***, (Tucker, 1979), zinc exposure to salmon, ***Salmo salar***, (Lorz and McPherson, 1976) and trout, ***Oncorhynchus mykiss***, (Watson and Beamish, 1980) increased the $Na^+$. $K^+$ ATPase activity in the gill tissues.

Aluminium metal in acidic waters has also been reported by Staurnes *et al.* (1993) to inhibit $Na^+$ $K^+$ ATPase activity in Atlantic salmon, ***Salmo salar***, and also in rainbow trout, ***Oncorhynchus mykiss***, (Staurnes *et al.*, 1984; Leivestad *et al.*, 1987; Reite and Staurnes, 1987; Rosseland *et al.*, 1992). Ganrot (1986) reported that aluminium interferes with basic biochemical functions, high energy metabolic and enzyme activities.

Inhibition of acid phosphatase activity was recorded in fish, ***Clarias lazera***, and ***Tilapia zillii***, by zinc exposure (Hilmy *et al.*, 1987) by cadmium

exposure in ***Sarotherodon mossambicus*** (Ruparelia ***et al.***, 1992) and in catfish ***Heteropneustes fossilis*** (Sastry and Subhadra, 1985) and in killifish ***Fundulus heteroclitus*** (Jackim ***et al.***, 1970). However, increase in acid phosphatase activity by cadmium exposure in bluegill sunfish ***Lepomis macrochirus*** (Versteeg and Giesy, 1986) and copper exposure in ***Oncorhynchus mykiss*** (Leland, 1983) was observed.

Among various enzymes ~~were~~ studied, much attention has been focussed on alkaline phosphatase (ALPase) by Yan and Chen (1986) and Gill ***et al.*** (1990,1991, 1992). Alkaline phosphatase is a polyfunctional enzyme which hydrolyses a broad class of phosphomonoester substrates and acts as a transphosphorylase at alkaline pH (Donachy ***et al.***, 1990; Blasco ***et al.***, 1993). The enzyme may also play an important role in the mineralization of the skeleton of aquatic animals (Donachy ***et al.***, 1990; Olsen ***et al.***, 1991).

Determination of 'alkaline' serum phosphatase is of great importance in the differential diagnosis of many diseases in man (Linhardt and Walter, 1965). According to Boge ***et al.*** (1992) in ecotoxicology, alkaline phosphatase could serve as a good indicator of intoxication because of its sensitivity to metabolic salts. Increased activity of alkaline phosphatase was recorded in the liver of freshwater fish, ***Sarotherodon mossambicus*** and in alevins of brook trout, ***Salvelinus fontinalis*** ~~by~~ in cadmium exposure (Ruparelia ***et al.***, 1992; Christensen ***et al.***, 1975), ~~respectively~~. ALP activity was significantly increased when red sea bream ***Chrysophrys major*** were exposed to zinc (Haya and Waiwood, 1983).

Joshi and Desai (1981), Casillas and Ames (1986), Casillas and Myers (1989) and Folmar *et al.* (1993) found higher alkaline phosphatase activity in fishes exposed to toxicants. According to Lan *et al.* (1995) alkaline phosphatase activity was increased at lower concentrations of copper exposure whereas, higher concentrations of copper significantly inhibited the activity of the ALP enzyme in the liver of red sea bream, ***Chrysophrys major***. The authors further have shown that the effect of selenium and chromium on ALPase activity was minimal in the liver of red sea bream, ***Chrysophrys major***. Boge *et al.* (1988, 1992) reported that alkaline phosphatase enzyme is sensitive to hexavalent chromium exposure in trout, ***Oncorhynchus mykiss***. Numerous other metallic cations, which are normal constituents of the tissues such as zinc, cobalt and magnesium also affected the alkaline phosphatase activity (Tardivel *et al.*, 1987). Bamberger *et al.* (1968) noticed inhibition of alkaline phosphatase by beryllium and aluminium.

Metals are considered serious materials, as they have long residence times in living organisms (Leatherland and Burton, 1974). Metal ions may damage organisms at the cellular levels by several possible mechanisms which include disruption of cellular structure or interfere with metabolic function by substituting metabolic cofactors; the metal ions may be electrostatically attracted to negatively charged cell membranes competing for or interfering with transport function or bringing about structural alteration (Tucker, 1979). It is reported that aluminium ions interferes unlimited numbers of biochemical reactions in the cells (Ganrot, 1986).

The foregoing review of literature reveals that not much work has been done on the effect of aluminium toxicity on enzyme activity of fish, ***Cyprinus carpio*** var. ***communis***. Hence, in the present study the nature and degree of enzyme response in ***Cyprinus carpio*** var. ***communis*** to aluminium toxicity in neutral waters was investigated.

# MATERIAL AND METHODS

$Na^+$. $K^+$ ATPase activity in gills and plasma and acid and alkaline phosphatase activity in plasma of control and experimental fishes were studied.

## PREPARATION OF SAMPLE

The blood was collected from live fish of control and experimental groups for enzyme assay. The blood was drawn from the heart region by cardiac puncture using a syringe, pre-rinsed with ~~heparin~~, ~~an~~ the anticoagulant heparin and it was transferred into small vials. The sample was centrifuged at 9000 rpm for 5 ~~mts~~ min. This left a clear yellow fluid, the plasma, which was used for the determination of $Na^+$ $K^+$ ATPase, acid phosphatase and alkaline phosphatase.

### Gills

The gills were separated from the control and experimental fish, and 200 mg tissue from each was weighed. They were homogenised with 1 ml of 0.1 M. Tris-HCl buffer (pH 7.5) using a teflon homogeniser, and then centrifuged at 10,000 rpm at $4^0$C for 15 min. The supernatant was used for $Na^+$ $K^+$ ATPase activity studies in gills.

## ESTIMATION OF ADENOSINE TRIPHOSPHATASE (ATPase)

### SODIUM AND POTASSIUM ATPase

The specific activities of $Na^+$, $K^+$ ATPase were assayed following the method of Shiosaka ***et al.*** (1971).

**Principle**

Adenosine triphosphatase catalyses the conversion of ATP to ADP. During this conversion, one molecule of phosphorus is liberated.

$$\text{Adenosine triphosphate} \xrightarrow{\text{ATPase}} \text{Adenosine diphosphate} + \text{Pi}$$

The inorganic phosphorus liberated was estimated following the method of Fiske and Subbarow (1925). In this method, the protein is precipitated with trichloroacetic acid. The protein free filtrate is treated with aluminium molybdate solution and the phosphoric acid formed is reduced by the addition of 1-amino-2-napthol 4-sulphonic acid (ANSA) reagent to produce the blue colour. The intensity of the colour is proportional to the amount of phosphorus present.

**Reagents**

**1. Tris-HCl Buffer Solution (0.1M; pH 7.5)**

12.10 g of Tris hydroxy methyl amino methane was dissolved in 80.00 ml of 1N HCl and made upto 1 L with distilled water. The pH was adjusted to 7.5.

**2. 0.02M Adenosine Triphosphate Solution**

11.023 mg of free ATP was dissolved in 1.00 ml of distilled water (w/v). This solution was prepared fresh at the time of use.

**3. Ammonium Molybdate Solution**

25.00 g of ammonium molybdate was dissolved in 100.00 ml (w/v) of distilled water. In 1 L flask, 300.00 ml of 10 N Sulphuric acid (83.10 ml of concentrated sulphuric acid of 36 N was made up to 300.00 ml with distilled water) was taken, and to this, ammonium molybdate was added and made upto 1 L with distilled water.

**4. 5% Trichloro Acetic Acid Solution**

5.00 g of TCA was dissolved in 100.00 ml of distilled water (w/v).

**5. 15% Sodium Bisulphite Solution**

30.00 g of sodium bisulphite was dissolved in 200.00 ml of distilled water (w/v). If the solution is turbid, it is allowed to stand for several days and filtered.

**6. 20% Sodium Sulphite Solution**

20.00 g of anhydrous sodium sulphite was dissolved in 100.00 ml of distilled water (w/v).

**7. ANSA Reagent (1-amino-2-naphthol-4-sulphonic acid)**

0.50 g of ANSA was dissolved in 195.00 ml of 15% sodium bisulphite solution in a brown bottle. To this, 5.00 ml of 20% sodium sulphite solution was added and mixed well. This was prepared freshly for every use.

**8. 100 mM Sodium Chloride Solution**

584.00 mg of NaCl was dissolved in 100.00 ml of distilled water (w/v).

**9. 100 mM Potassium Chloride Solution**

746.00 mg of KCl was dissolved in 100.00 ml of distilled water (w/v).

**Standard preparation**

0.315 g of pure monopotassium phosphate was dissolved in distilled water and transferred quantitatively to 1L volumetric flask. Then 10.00 ml of 10 N sulphuric acid was added to this and diluted to the mark with distilled water and mixed well. This solution contains 0.40 mg of phosphorus in 5.00 ml.

**Procedure for standard graph preparation**

From the standard solution, 0.10 ml, 0.20 ml, 0.30 ml, 0.40 ml, 0.50 ml, 0.60 ml, 0.70 ml, 0.80 ml, 0.90 ml, and 1.00 ml of the solution were taken, respectively in 10 test tubes, which corresponds to their respective concentrations of 8,16,24,32,40,48,56,64,72 and 80 µg of phosphorus. To each test tube, distilled water was added and made upto 1.00 ml. Then 1.00 ml of ammonium molybdate and 0.40 ml of ANSA were added to each tube and the blue colour formed was immediately read against blank at 680 nm using Spectronic - 20D (Milton Roy, USA) and the respective OD values were noted.

For plotting the standard graph, the OD values were taken on the Y axis and the concentration of phosphorus along the X axis. Different concentrations and

their respective OD values were plotted and points obtained were joined by a straight line.

**Procedure for enzyme assay**

Clean test tubes were taken and labelled 'Blank' and 'Test'. To each tube, 0.30 ml of Tris-HCl buffer (pH 7.5), 0.10 ml of 0.02 M ATP, 0.10 ml of 100 mM NaCl and 0.10 ml of KCl (for $Na^+$, $K^+$-ATPase) were added.

Then, 0.10 ml of distilled water was added to blank, and to the test 0.10 ml of tissue extract/plasma was added to the respective tubes. The reaction mixture was mixed and incubated in water bath at $37^0$C for 15 min. After the incubation period, the reaction was terminated with 2.0 ml of 5% TCA. The tubes were kept at $4^0$C for 30 min. and centrifuged for 5 min. at 500 rpm.

To the supernatant, 1.00 ml of ammonium molybdate and 0.40 ml of ANSA reagent were added and allowed to stand for 10 min. at room temperature. The intensity of the blue colour developed was read at 680 nm against a reagent blank using spectrophotometer (Spectronic 20D, Milton Roy, USA). Suitable standards were also run through each batch of assays. The enzyme activity was expressed in terms of micrograms/microlitres of inorganic phosphorus formed per hour per gram of tissue/per litre of plasma.

**Calculation**

**Step I**

The OD values of both control and experimental samples were marked on the Y axis of the standard graph and it was extrapolated to the corresponding phosphorus concentration on the X axis.

**Step II**

The $Na^+$, $K^+$ ATPase enzyme activity was calculated as per the following formula

**For gills**

$$\text{Activity} = \text{Concentration of phosphorus} \times \frac{\text{Total volume taken for homogenisation}}{\text{Volume taken for analysis}} \times 4 \times \frac{1000}{\text{weight of tissue taken}} \times \frac{1}{1000}$$

expressed in µg/h/g tissue.

**For plasma**

$$\text{Activity} = \text{Concentration of Phosphorus} \times \text{Volume taken for analysis} \times 4 \times \frac{1}{1000}$$

expressed in µl/h/L of plasma.

**ESTIMATION OF ACID PHOSPHATASE**

Acid phosphatase activity was estimated by the method of Modified King's method of King and Jagatheesan (1959) using Diagnostic Reagent Kit supplied by Stangen Immuno diagnostics, Hyderabad, India.

**Principle**

Serum ACP hydrolyzes disodium phenyl phosphate to phenol and disodium phosphate at pH 5.0. The phenol so formed reacts with 4 aminoantipyrine in alkaline medium in the presence of oxidising agent potassium ferricyanide to form red coloured complex whose absorbance is proportional to the enzyme activity.

$$\text{Disodium phenyl phosphate} + H_2O \xrightarrow[\text{pH 5.0}]{\text{ACP}} \text{Phenol} + \text{Disodium Hydrogen phosphate}$$

$$\text{Phenol} + \text{amino antipyrine} \xrightarrow[\text{Potassium Ferricyanide}]{\text{Alkaline medium}} \text{Red coloured complex}$$

**Reagents**

1. Substrate
2. Buffer solution pH 5.0
3. Color reagent
4. Phenol standard 10 KA units

**Reagent preparation**

One vial of substrate (1) was reconstituted with 5.5 ml of buffer solution (2) and mixed well.

**Procedure**

Four test tubes were taken and named as Blank (B), standard (S), Control (C), and test (T). To each tube 1.0 ml of buffered substrate was added. Then

3.2 ml , 3.1ml, 3.0 ml and 3.0 ml of deionized water was added to the test tubes blank (B), standard (S), control (C), and test (T) respectively. They were mixed well and incubated at $37^0$C for 3 min. Then 0.2 ml of plasma was added to the test (T) tube and 0.1 ml of phenol standard was added to the tube standard (S). The above solutions were mixed well and incubated at $37^0$C for 60 min. Then 2.0 ml of plasma was added to control tube (C). The above reagents were mixed well and the absorbance of Blank (B), standard (S), control (C) and test (T) against distilled water were measured in spectrophotometer at 510 nm.

**Calculation**

$$\text{ACP activity in KA Units} = \frac{\text{A of (T) - A of (C)}}{\text{A of (S) - A of (B)}} \times 5$$

## ESTIMATION OF ALKALINE PHOSPHATASE

Alkaline phosphatase activity was estimated by Kind and King's (1954) Method using Diagnostic Reagent Kit supplied by Stangen immunodiagnostics, Hyderabad, India.

**Principle**

Serum ALP hydrolyses phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10.0. The phenol so formed reacts with 4.amino antipyrine in alkaline medium in presence of oxidising agent potassium ferricyanide to form a red colored complex, whose absorbance is proportional to the enzyme activity.

$$\text{Disodium phenyl phosphate} + H_2O \xrightarrow[\text{pH 10.0}]{\text{ALP}} \text{Phenol} + \text{Disodium Hydrogen Phosphate}$$

$$\text{Phenol} + \text{4amino antipyrine} \xrightarrow[\text{Potassium ferricyanide}]{\text{Alkaline medium}} \text{Red coloured complex}$$

**Reagents**

1. Buffered substrate
2. Color reagent
3. Phenol standard (10 KA units)

**Reagent preparation**

Buffered substrate was reconstituted with 4.5 ml of distilled water and mixed well.

**Procedure**

Four test tubes *viz*., Blank (B), standard (S), control (C) and test (T) were taken and to each test tube 1.0 ml of working buffered substrate was added. Then 3.1 ml deionized water was added to the Blank (B), and 3.0 ml of deionised water was added to the rest of three tubes. The samples were incubated for 3 ~~mts~~ min. at 37$^0$C. Then 0.1 ml of (plasma) was added to test (T) and 0.1 ml of phenol standard (s) was added to the standard (S). Then 0.1 ml of plasma was added to the control (C) tube. Again, the samples were incubated for 15 min at 37$^0$C and 2.0 ml of color reagent was added to each of four tubes. The above samples were mixed well after each addition of

reagent and absorbance was measured for Blank (B), standard (S), control (C) and test (T) against deionised water using spectrophotometer at 510 nm.

**Calculation**

$$\text{ALP in KA units} = \frac{\text{A of (T) - A of (C)}}{\text{A of (S) - A of (B)}} \times 10$$

# RESULTS

Table 36 and Fig.21 depict the changes in the enzyme activities of $Na^+$ $K^+$ ATPase in gills and plasma and acid and alkaline phosphatase in plasma of ***Cyprinus carpio*** var. ***communis*** exposed to lethal concentration of aluminium sulphate. In gills, the $Na^+$ $K^+$ ATPase activity decreased significantly showing 35.47% whereas in plasma it increased by 6.35% ~~than~~ that ~~of their~~ relative to the controls. Both acid and alkaline phosphatase activity increased in experimental fish, showing a 144.64% and 387.67% respectively.

Table 37 and Fig. 22 gives the data on the changes in $Na^+$ $K^+$ ATPase activity in the gills of fish, ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium. The enzyme activity decreased to 3.52% after 7th day in treated fish. The declining trend continued upto middle of the experiment recording 18.64% and 46.90% decrease on 14th and 21st day, respectively. After 21st day, the enzyme activity gradually recovered recording a per cent decline of 16.00% and 8.67% at the end of 25th and 35th day respectively.

Table 38 represents the DMRT data for $Na^+$ $K^+$ ATPase activity of gills in ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium. The $Na^+$ $K^+$ ATPase activity of the treated group varied from that of the control on the 7th day. A similar trend was observed for all the other exposure periods.

Table 36 : $Na^+$ $K^+$ ATPase activity in the gills and plasma and plasma phosphatases activity of ***Cyprinus carpio*** var. ***communis*** exposed to acute aluminium sulphate toxicity.

| S .No | Parameters | Control | Experimental | Per cent change | Calculated ' t ' |
|---|---|---|---|---|---|
| 1 . | $Na^+ K^+$ ATPase. | | | | |
| | a,Gills (µg/h/gm) | 42.400±1.043 | 27.360± 0.888 | - 35.47 | 10.9815 * |
| | b.Plasma(µl/h/L) | 0.0239±0.007 | 0.0254±0.0007 | +6.35 | 1.4767 |
| 2 . | Acid phosphatase in KA units | 22.85± 0.527 | 55.900±1.174 | + 144.64 | 25.6748 * |
| 3 . | Alkaline phosphatase in KA units | 14.600± 0.176 | 71.200±1.476 | + 387.67 | 38.0899 * |

Values are mean ± S. E. of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control
Values are significant at 5% level
Degrees of freedom at 8 t 0.05 = 2.306.

Fig. 21. $Na^{+}K^{+}$ ATPase activity in gills and plasma and plasma phosphatase activity of ***Cyprinus carpio*** var. ***communis*** exposed to 24 h LC 50 concentration of aluminium sulphate toxicity.

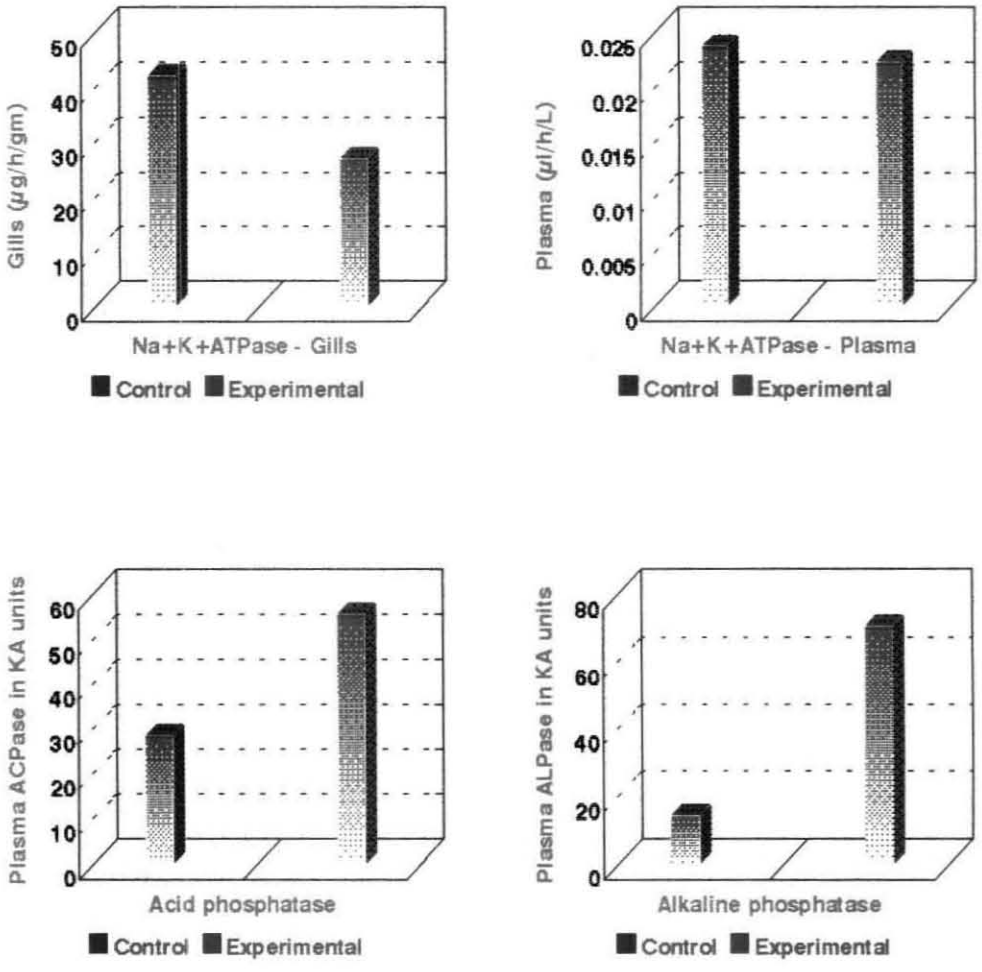

Gills (µg/h/gm)
50
40
30
20
10
0
Na+K+ATPase - Gills
Control
Experimental
Plasma (µl/h/L)
0.025
0.02
0.015
0.01
0.005
0
Na+K+ATPase - Plasma
Control
Experimental
Plasma ACPase in KA units
60
50
40
30
20
10
0
Acid phosphatase
Control
Experimental
Plasma ALPase in KA units
80
60
40
20
0
Alkaline phosphatase
Control
Experimental

**Fig. 21**

Table 37 : $Na^+ K^+$ ATPase activity in the gills of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Gill $Na^+ K^+$ ATPase activity µg/h/g tissue | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 42.000 ± 1.045 | 40.523 ± 1.258 | -3.52 |
| 14 | 36.320 ± 1.228 | 29.550 ± 0.707 | -18.64 |
| 21 | 36.160 ± 1.147 | 19.200 ± 0.872 | -46.90 |
| 28 | 40.000 ± 1.820 | 33.600 ± 1.054 | -16.00 |
| 35 | 42.400 ± 0.712 | 38.720 ± 1.504 | -8.67 |

Values are mean ± S . E . of five individual observations
- Denotes per cent decrease over control

Fig. 22. $Na^+K^+$ATPase activity in the gills of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

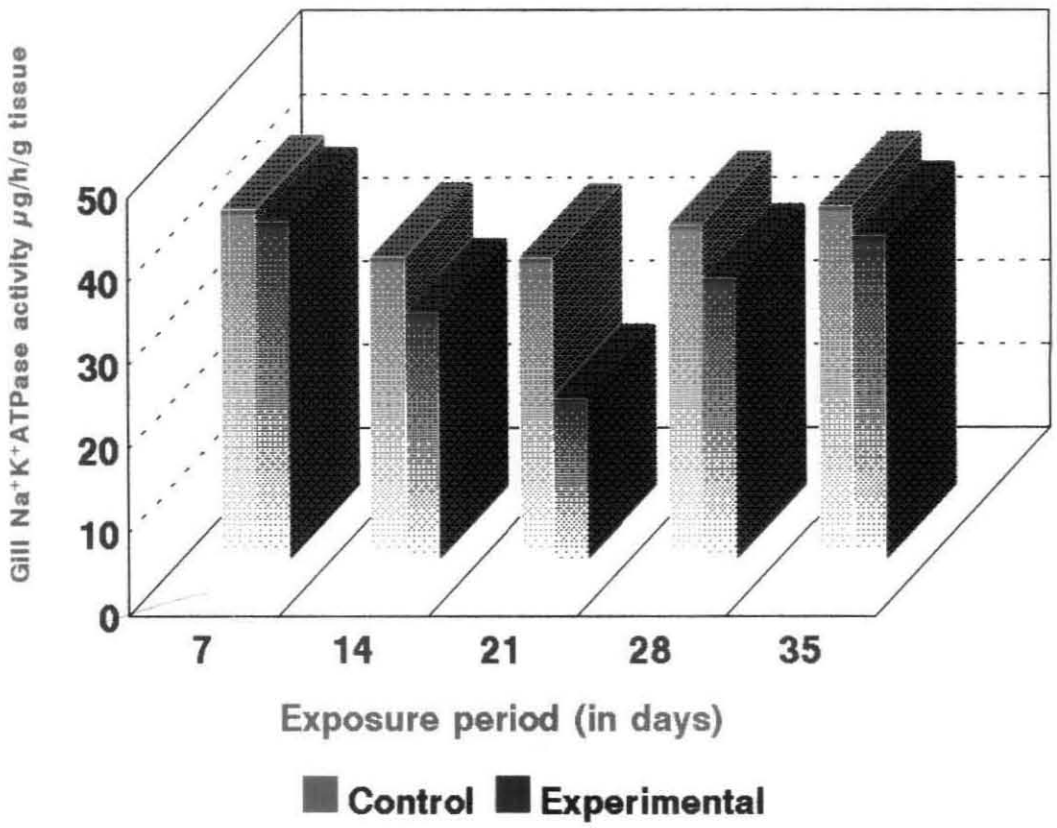

Gill Na+K+ATPase activity µg/h/g tissue
50
40
30
20
10
0
7
14
21
28
35
Exposure period (in days)
Control
Experimental

Fig. 22

Table 38 : DMRT table for $Na^+$ $K^+$ ATPase activity in the gills of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $42.000^{Ba}$ | $40.523^{Db}$ | $41.262^{IV}$ |
| 14 | $35.320^{Aa}$ | $29.550^{Bb}$ | $32.935^{II}$ |
| 21 | $36.160^{Aa}$ | $19.200^{Ab}$ | $27.680^{I}$ |
| 28 | $40.000^{Ba}$ | $33.600^{Cb}$ | $36.800^{III}$ |
| 35 | $42.400^{Ba}$ | $38.720^{Db}$ | $40.560^{IV}$ |
| Mean | $39.376^{\alpha}$ | $32.319^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at $F < 0.05$
CV = 7.379 %

When control group alone was analysed for varying exposure period, 7th, 28th and 35th day were on par with each other, but they varied significantly from that of the 14th and 21st which were also on par with each other.

When treatment groups alone were analysed 7th and 35th day were on par with each other but varied significantly from that of 14th, 21st and 28th day which also varied significantly among themselves. A similar trend was observed for period means. When treatment means were analysed irrespective of the exposure period, both the control and treatment significant from each other indicating the deleterious action of aluminium in enzyme activity in gills.

Table 39 and Fig. 23 show data on $Na^+$ $K^+$ ATPase activity in plasma of fish, ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium stress. $Na^+$ $K^+$ ATPase activity decreased by 15.38% and 27.07% after 7th and 14th day, respectively. However, after 21st day enzyme activity in plasma of treated fish suddenly increased by 83.10% than that of the control. Subsequently $Na^+$ $K^+$ ATPase activity reached to near normal value of control recording 1.28% decrease and 6.27% increase after 28th and 35th day, respectively.

Table 40 presents the DMRT data on changes in plasma $Na^+$ $K^+$ ATPase activity of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium concentration. The $Na^+$ $K^+$ ATPase level in the plasma of the experimental fish varied significantly at the end of the 7th, 14th and 21st day, from control whereas at the end of 28th and 35th day, the enzyme activity was on par with each other.

Table 39 : $Na^+$ $K^+$ ATPase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Plasma $Na^+$ $K^+$ ATPase µl/h/L | | |
|---|---|---|---|
| | Control | Treatment | Per cent change |
| 7 | 0.0234± 0.0006 | 0.0198± 0.0009 | -15.38 |
| 14 | 0.0229± 0.0010 | 0.0167± 0.0006 | -27.07 |
| 21 | 0.0227± 0.0010 | 0.0415 ± 0.0007 | +83.10 |
| 28 | 0.0234 ± 0.007 | 0.0231 ± 0.0006 | -1.28 |
| 35 | 0.0239± 0.0010 | 0.0254 ± 0.0010 | +6.27 |

Values are mean ± S . E . of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control

Fig. 23. $Na^+K^+$ATPase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

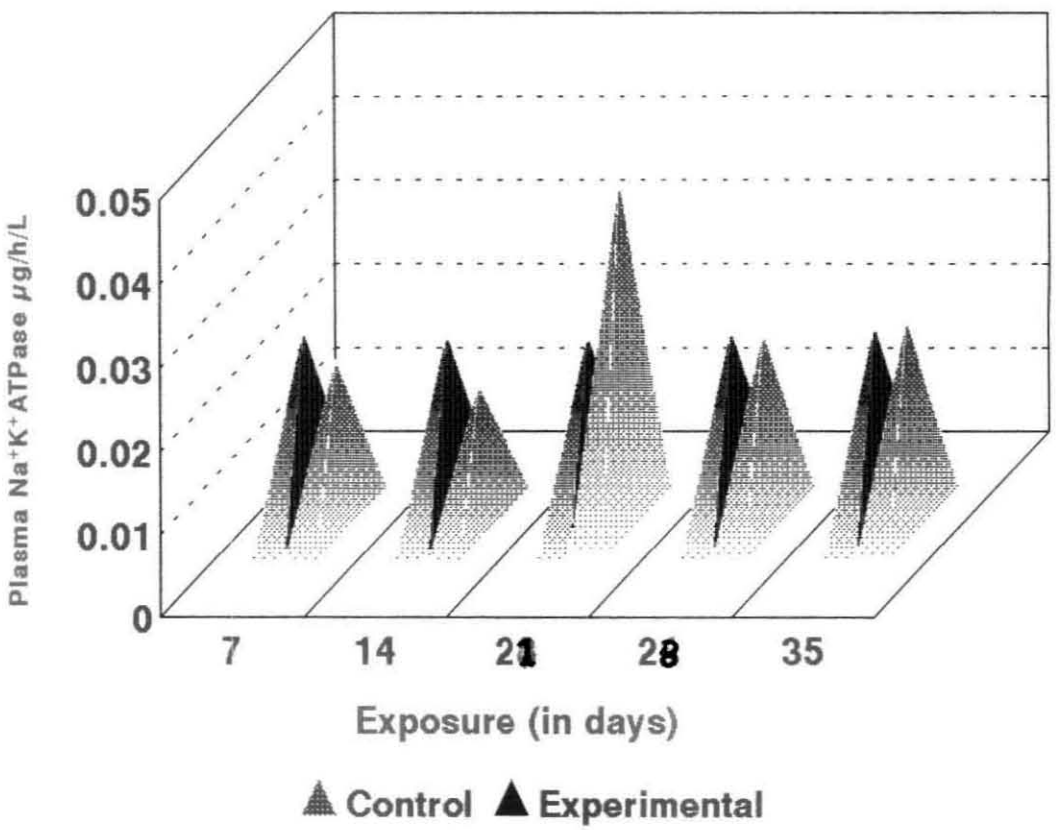

Plasma Na+K+ATPase µg/h/L
0.05
0.04
0.03
0.02
0.01
0
7
14
21
28
35
Exposure (in days)
Control
Experimental

Fig. 23

Table 40 : DMRT table for $Na^+$ $K^+$ ATPase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $0.0234^{Aa}$ | $0.0198^{Bb}$ | $0.0216^{I, II}$ |
| 14 | $0.0229^{Aa}$ | $0.0167^{Ab}$ | $0.0198^{I}$ |
| 21 | $0.0227^{Aa}$ | $0.0415^{Db}$ | $0.0321^{IV}$ |
| 28 | $0.0234^{Aa}$ | $0.0231^{Ca}$ | $0.0232^{II, III}$ |
| 35 | $0.0239^{Aa}$ | $0.0254^{Ca}$ | $0.0246^{III}$ |
| Mean | $0.0232^{\alpha}$ | $0.0253^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV = 8.748 %

Analysis of control group alone for varying periods of exposure showed that the $Na^+$ $K^+$ ATPase activities were on par at all the periods. Analysis of treatment group alone revealed only 28th and 35th day was on par with each other but varied significantly from that of 7th, 14th and 21st days, which also varied significantly among themselves.

Analysis of period means, irrespective of treatment means revealed that 7th and 14th, 7th and 28th and 28th and 35th day were on par with each other, while the rest of the combinations varied significantly. Analysis of treatment means irrespective of the exposure period also showed that the treatment group varied significantly than the control.

Changes in the plasma acid phosphatase activity of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal aluminium sulphate were given in Table 41 and Fig 24. The enzyme activity increased to 95.99% in the experimental fish after 7th day treatment. However, after 14th day, it decreased to 19.79%. In the following weeks, again the enzyme activity substantially increased showing 79.10%, 222.90% and 285.51% after 21st, 28th and 35th day, respectively.

Table 42 gives the data of DMRT analysis on changes in the plasma of acid phosphatase activity. ACP activity varied significantly in the treatment group at all the exposure periods than the control.

When control group alone was tested 7th and 14th, 14th and 21st, 28th and 35th day were on par with each other while 7th, 21st and 28th day, 7th, 21st and 35th day varied significantly among themselves. Analysis of treatment group alone for

Table 41 : Acid phosphatase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

| Exposure period (in days) | Plasma acid phosphatase in KA units | | |
|---|---|---|---|
| | Control | Treatment | Per cent change |
| 7 | 24.950 ± 0.590 | 48.900 ± 1.064 | +95.99 |
| 14 | 27.265 ± 0.375 | 21.860 ± 0.825 | -19.79 |
| 21 | 28.590 ± 1.205 | 51.210 ± 0.839 | +79.10 |
| 28 | 21.400 ± 0.744 | 69.110 ± 0.637 | +222.9 |
| 35 | 21.400 ± 0.450 | 82.500 ± 0.951 | +285.51 |

Values are means ± S . E . of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control

Fig. 24. Acid phosphatase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

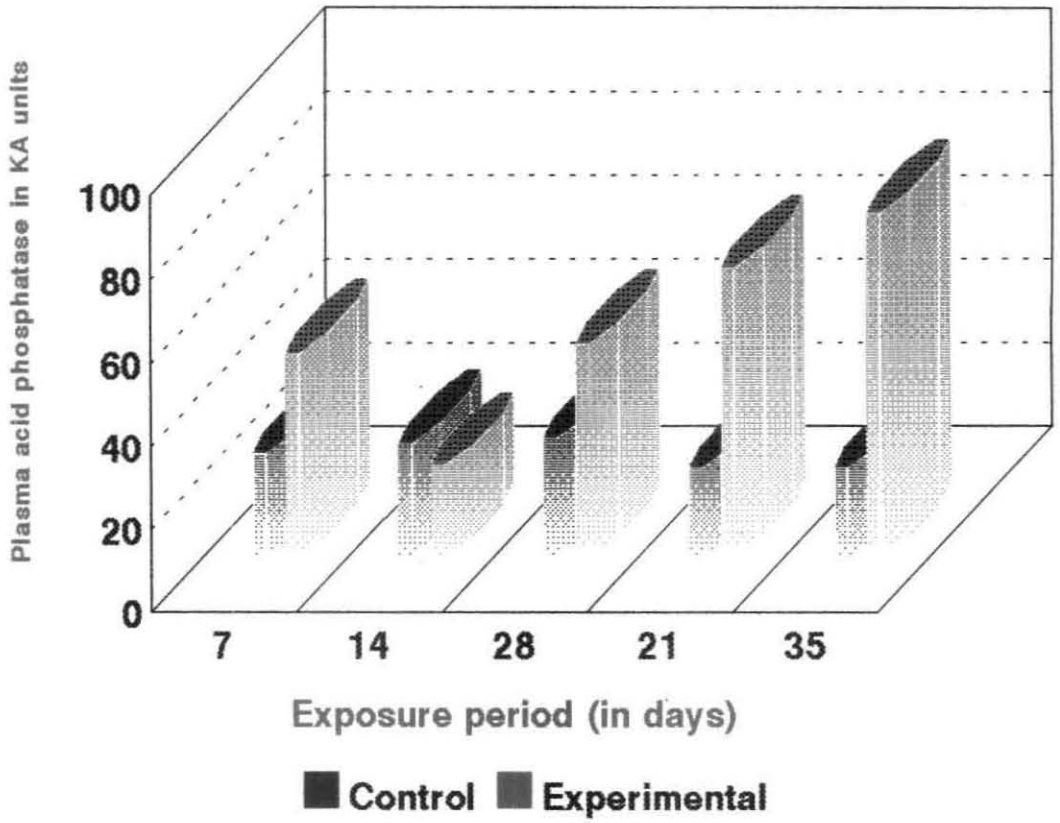

Plasma acid phosphatase in KA units
100
80
60
40
20
0
7
14
28
21
35
Exposure period (in days)
Control
Experimental

Fig. 24

Table 42 : DMRT table for acid phosphatase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | 24.950 $^{Ba}$ | 48.900 $^{Cb}$ | 36.93 $^{II}$ |
| 14 | 27.265 $^{BCa}$ | 21.860 $^{Ab}$ | 24.56 $^{I}$ |
| 21 | 28.590 $^{Ca}$ | 51.210 $^{Bb}$ | 39.90 $^{III}$ |
| 28 | 21.400 $^{Aa}$ | 69.110 $^{Db}$ | 45.26 $^{IV}$ |
| 35 | 21.400 $^{Aa}$ | 82.500 $^{Eb}$ | 51.95 $^{V}$ |
| Mean | 24.72$^{\alpha}$ | 54.72$^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represent comparison of period means
Degree of freedom at F <0.05
CV % = 4.548 %

varying periods of exposure revealed that all the exposure periods varied significantly among themselves.

Analysis of period means irrespective of the treatments also depicted a similar trend like that of the treatment groups. When treatment means were analysed irrespective of the exposure period, both the control and treatment groups varied significantly between themselves.

Table 43 and Fig. 25 illustrate the changes occurred in the alkaline phosphatase level in the plasma of fish by the exposure of sublethal concentration of aluminium sulphate. Alkaline phosphatase activity decreased to 54.33% at the end of 7th day, after which the activity gradually recovered showing 27.70% at the end of 14th day then the value reached to near normal by registering 4.61% increase after 21st day. Then the enzyme activity decreased showing 51.77% and 29.89% after 28th and 35th day, respectively.

Table 44 gives the data of DMRT analysis of plasma alkaline phosphatase activity of fish to sublethal aluminium exposure. At the end of the 7th both the control and treatment groups varied significantly. A similar trend was observed for 14th and 35th day. On the other hand, at the end of 21st, 28th day, both the control and treatment groups were on par with each other.

When control group alone was tested, 7th and 21st days, 14th, 28th and 35th day, 21st and 35th days were on par with each other while the rest of the combinations varied significantly. When treatment groups alone were analysed 14th

Table 43 : Alkaline phosphatase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Plasma alkaline phosphatase activity in KA units | | Per cent change |
|---|---|---|---|
| | Control | Treatment | |
| 7 | 16.600 ± 0.698 | 7.580 ± 0.308 | -54.33 |
| 14 | 14.800 ± 0.853 | 10.700 ± 0.491 | -27.70 |
| 21 | 15.200 ± 0.455 | 15.900 ± 0.420 | +4.61 |
| 28 | 14.100 ± 0.487 | 13.670 ± 0.830 | -51.77 |
| 35 | 14.700 ± 0.302 | 10.306 ± 0.804 | -29.89 |

Values are means ± S . E . of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control

Fig. 25. Alkaline phosphatase activity in the plasma of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

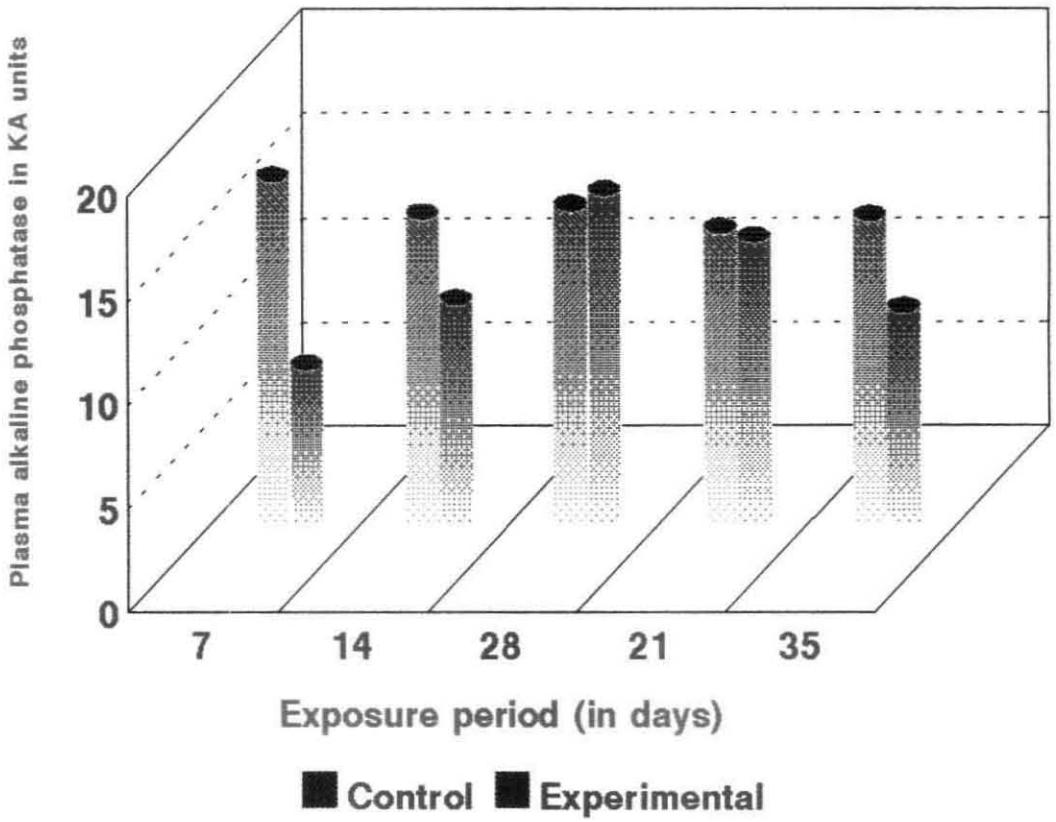

Plasma alkaline phosphatase in KA units
20
15
10
5
0
7
14
28
21
35
Exposure period (in days)
Control
Experimental

Fig. 25

Table 44 : DMRT table for plasma alkaline phosphatase activity in plasma of ***Cyprinus carpio*** var. ***communis*** exposed to aluminium sulphate toxicity for varying periods.

| Exposure period (in days) | Control | Treatment | Mean |
|---|---|---|---|
| 7 | $16.600^{Ca}$ | $7.580^{Ab}$ | $12.090^{I}$ |
| 14 | $14.800^{Aa}$ | $10.700^{Bb}$ | $12.750^{I, II}$ |
| 21 | $15.200^{BCa}$ | $15.900^{Da}$ | $15.550^{III}$ |
| 28 | $14.100^{Aa}$ | $13.670^{Ca}$ | $13.890^{II}$ |
| 35 | $14.700^{ABa}$ | $10.306^{Bb}$ | $12.500^{I}$ |
| Mean | $15.080^{\alpha}$ | $11.630^{\beta}$ | |

Capital letters represent comparison of column means (Different periods for a particular treatment)
Small letters represent comparison of row means (Different treatments for a particular period)
Greek letters represent comparison of treatment means
Roman letters represented comparison of period means
Degree of freedom at F <0.05
CV = 10.042 %

and 35th day were on par with each other but varied significantly from that of 7th, 21st and 28th days, which also varied significantly among themselves.

When period means were analysed irrespective of the treatment, 7th, 14th and 35th day and 14th and 28th were on par with each other, while 7th, 21st and 28th and 35th day varied significantly among each other. When treatment means were analysed irrespective of the exposure periods, both of them varied significantly among each other thus indicating that aluminium imparts toxicity on alkaline phosphatase activity in the plasma of fish ***Cyprinus carpio*** var. ***communis***.

# DISCUSSION

Metals may alter enzyme activity in animals in several ways (Kench, 1972; Verma *et al.*, 1983). They may bind to macromolecules inside the cells particularly with enzymes (Vallee and Ulmer 1972; Britten and Blank, 1973) or change the concentrations of cofactors or reactants by altering membrane permeability and indirectly affecting enzyme activity (Tucker and Matte, 1980).

According to Mayer *et al.* (1992), basically there are four different processes that describe the responses of enzymes to specific or non-specific chemical stress. They are: 1. direct enzyme inhibition 2. enzyme induction by specific classes of chemicals 3. elevation of serum enzymes *via* tissue damage and 4. alterations in enzyme activity as a result of changes in metabolic pathways or fluxes. The author further added that enzyme activity is generally regulated such that specific substrates or entire pathways may be homeostatically adjusted to compensate for endogenous or exogenous changes.

$Na^+$ $K^+$ ATPase has been reviewed and assessed as a potentially useful indicator of pollution stress in aquatic animals (Haya and Waiwood, 1983; Torreblanca *et al.*, 1989). Inhibition or stimulation of $Na^+$ $K^+$ ATPase activity could be expected to have metabolic or ionic effect in fishes in relation to osmoregulation as stated by Verma *et al.* (1983).

Heavy metals inhibit $Na^+$ $K^+$ ATPase activity and interfere with osmoregulatory mechanisms of aquatic organisms (Schmidt-Nielson, 1974; Dhavale

*et al.*, 1988). According to Walton (1973), lead hydrolyses ATP thus changing the concentration of a component of the ATPase reaction and indirectly alters the enzyme activity. Such inhibition on $Na^+$ $K^+$ ATPase is reported in mammalian tissues by lead and cadmium (Hasan *et al.*, 1967; Cross *et al.*, 1970; Cardona *et al.*, 1971). Tucker and Matte (1980) observed inhibition of $Na^+$ $K^+$ ATPase in the gills of rock crab, ***Cancer irroratus*** after exposure to cadmium and lead. The authors described it as a result of the replacement of a normal co-factor, possibly $Mg^{2+}$, in ATPases by the metal ions leading to alteration in enzyme activity. The above view is further supported by Tucker (1979) ~~by cadmium exposure~~ exposed to cadmium in the gills of lobster, ***Homarus americanus***.

In agreement with this, copper ~~declined~~ decreased the $Na^+$ $K^+$ ATPase activity in the gills of coho salmon, ***Oncorhynchus kisutch***, (Lorz and McPherson, 1976). Gill $Na^+$ $K^+$ ATPase activity was found to be inhibited in chinook salmon, ***Oncorhynchus ashawytscha***, by copper exposure as reported by Beckman and Zaugg (1988). The authors suggested that copper damages 'chloride cell' where $Na^+$ $K^+$ ATPase is located and impaired its function and the enzyme was inactivated by the direct inhibition of metals.

Rifkin (1965) and Folbergrova and Mares (1987) reported inhibition of $Na^+$ $K^+$ ATPase by vanadium by binding to the phosphate site on the enzyme located inside the cell. Shah *et al.* (1996) also recorded low level of $Na^+$ $K^+$ ATPase activity in the brain and liver tissues of ***Clarias batrachus*** by vanadium.

Kuhnert and Kuhnert (1976) reported decrease in $Na^+$ $K^+$ ATPase activity in many tissues of rainbow trout, ***Oncorhynchus mykiss***, by chromium

exposure. The authors stated that the degree of enzyme inhibition by chromium is directly related to ~~the~~ ~~tissues~~ of the various tissues. susceptibility. In support of this Fromm and Stokes (1962) observed high water content in tissues of fish exposed to chromium, which may be due to inhibition of kidney $Na^+$ $K^+$ ATPase by chromium exposure. Kuhnert and Kuhnert (1976) suggested that inhibition of enzyme activity by chromium may be brought about by the binding of trivalent chromium to protein. Gray and Sterling (1950) and Grogin and Oppenteimer (1955) suggested that hexavalent chromium may bind to protein ***in vivo*** after reduction to the trivalent state.

According to Panchalingam ***et al.*** (1991) aluminium toxicity is expressed by its ability to bind phosphate, ATP, or another functionally related biological compound containing phosphorus. Hence, ATP forms stronger complexes with $AL^{3+}$ than with $Mg^{2+}$ and the $Al^{3+}$ ATP complex acts as a strong inhibitor to $Mg^{2+}$ ATP as indicated by Ganrot (1986).

The possibility of direct inhibition of enzyme by aluminium also exists as Al has been shown to accumulate in the chloride cells of both brown trout, ***Salmo trutta*** and rainbow trout, ***Oncorhynchus mykiss***, when kept in Al rich acid water (Karlsson-Norrgren ***et al.***, 1986a, 1986b; Youson and Neville, 1987). In brook trout, ***Salvelinus fontinalis*** ~~also~~ aluminium has been found to accelerate the turnover and degeneration of the chloride cells (Chevalier ***et al.***, 1985). Since the high gill activity of $Na^+$ $K^+$ ATPase in smolts of Atlantic salmon, ***Salmo salar***, is localised in the chloride cells, such degeneration of these cells could have contributed to the low gill $Na^+$ $K^+$ ATPase activity of the aluminium exposed fish (Staurnes ***et al.***, 1993).

In the present study, the inhibition of $Na^+$ $K^+$ ATPase activity in the gills of fish ***Cyprinus carpio*** var. ***communis*** may be due to direct inhibition of enzyme or replacement of normal co-factor $Mg^{2+}$ ions or 'chloride cell' damage or by binding of the metal ion with the phosphate groups of enzyme recalling the observations of the above authors.

The increase in $Na^+$ $K^+$ ATPase activity may be due to 'hormesis' in fish as pointed out by Samsons and Marten (1981) and Yang and Randall (1996). The authors defined the term 'hormesis' as an over compensation to some inhibitory challenges. Thurberg ***et al.*** (1977) noted a statistically significant increase in ATPase activity and gill tissue respiration in lobsters, ***Homarus americanus***, exposed to cadmium which may be due to increased metabolic activity within gill tissue, rather than being a direct effect of cadmium on the enzyme.

Gallis ***et al.*** (1979) and Towle (1981) suggested that high $Na^+$ $K^+$ ATPase activity in gills of the teleosts appeared to provide an adaptive mechanism to support the increased $Na^+$ uptake, required in the dilute freshwater environments. In the present study the increase in the plasma $Na^+$ $K^+$ ATPase activity after the 21st day could be described as a form of 'hormesis' as reported by the above authors.

The xenobiotic can interact directly with the enzyme or alter $Na^+$ $K^+$ ATPase activity due to disruption of energy producing metabolic pathways (Watson and Beamish, 1980; Yadwad ***et al.***, 1990). ATP stores are reduced by increased energy demand in response to a physiologically stressful condition. Since $Na^+$ $K^+$ ATPase turnover rate is extremely rapid, $Na^+$ $K^+$ ATPase would be sensitive to factors

affecting protein biosynthetic pathways (Haya and Waiwood, 1983). A ~~The~~ high turnover of ATPase in plasma in the present study as evident from the peak activity occurred in the middle of the exposure period, was associated with decreased plasma protein.

According to Mayer ***et al.*** (1992) serum enzyme concentration may increase from ~~either of~~ the following: 1. enzyme leakage from a cell with a damaged cell membrane 2. increased enzyme production and leakage from the cell and 3. decreased enzyme clearance from the blood.

Chemical and non-chemical stressors can lead to release of cellular enzymes possibly due to localised ischemia, depletion of nucleotides and high energy phosphates or changes in membrane potential (Highman ***et al.***, 1965; Wilkinson, 1978; Cole and Palmer, 1979; Freedel ***et al.***, 1979; Rattner ***et al.***, 1983). Metals have also been shown to reduce membrane stability possibly leading to enzyme leakage (Moore and Stebbing, 1976; Leland, 1983; Versteeg, 1985; Versteeg and Giesy 1986). Increased serum lysosomal enzymes is an indication of toxicity which may be due to increased enzyme production, decreased lysosomal stability or tissue damage as opined by Mayer ***et al.*** (1992).

According to Versteeg and Giesy (1986), sublethal cadmium exposure to bluegill sunfish, ***Lepomis macrochirus***, caused significant increase in acid phosphatase level in plasma. The author stated that the high activity of plasma ACP may be due to action of metal directly or indirectly on the tissue, altering lysosomal

membranes and rendering them more permeable to enzymes. Dubale and Shah (1981) made a similar observation in ***Channa punctatus*** during sublethal cadmium exposure.

Leland (1983) and Ramesh ***et al.*** (1993) noted increase in ACP level in juvenile rainbow trout, ***Oncorhynchus mykiss*** and in ***Oreochromis mossambicus*** after copper and nickel exposure, respectively. The authors ascribed it to the necrotic changes in the tissues and lysosomal membrane.

Arillo ***et al.*** (1981) have proposed three hypotheses for the lysosomal membrane damages by ammonia in trout liver tissue as follows: (1) Cationic groups of lysosomal enzymes form electrostatic bonds with the intra lysosomal matrix. Exogenous cations compete for anionic sites, displacing the lysosomal enzymes and increasing the ease with which they pass through the lysosomal membrane. (2) Toxicant accumulation in the lysosomes result in an osmotic gradient, leading to lysosomal swelling, which results in increased lysosomal lability. (3) Endogenous hormones elicited by the general stress response would increase lysosomal membrane lability.

The hypothesis of cationic displacement and metal induced swelling have been investigated for mercury in mouse liver lysosomes (Verity and Reith, 1967) and for copper in rat liver lysosomes (Chvapil ***et al.***, 1972). These authors suggested that metals which form redox systems may catalyse lipid peroxidation and cause membrane damage. A similar sequences of events might have occurred by aluminium toxicity, and it might have caused lysosomal membrane damage and in turn elevated

the ACP activity in the plasma of fish, ***Cyprinus carpio*** var. ***communis***, during acute and sublethal exposure in the present study.

Ruparelia ***et al***. (1992) reported a significant increase in the alkaline phosphatase level after cadmium exposure in the liver of freshwater fish ***Sarotherodon mossambicus***, which is dose and time dependent. Christensen (1975) made similar observation in brook trout, ***Salvelinus fontinalis***. According to Copious Peereboom ***et al***. (1979) the rise in ALP activity in liver of rats could be attributed to enzyme induction. Latner (1975) opined that the induction of hyperglycemia may increase the level of ALP activity which is further supported by the report of Ruparelia ***et al***. (1992) who found that the Cd intoxicated fish exhibited rise in ALP activity with reduction in glycogen content in liver.

ALP activity increase by nickel stress was reported by Ramesh ***et al***. (1993). The authors stated that nickel might have led to release of corticosteroids which resulted in increased adrenal secretion which in turn might have affected liver and kidney enzyme profiles. The above observations were supported by the findings of Murphy ***et al***. (1964) and Joshi and Desai (1981).

According to Ganrot, (1986) within cells, $Al^{3+}$ accumulates first in the lysosomes and then is slowly transferred to the nucleus of the cell. The author, further reported that the toxicity effect could be even greater at a more neutral pH if $Al^{3+}$ has adequate time to bind in sufficient quantities to the membranes.

Lieberherr ***et al***. (1982) showed that in human skeletal tissue acid and alkaline phosphatases are activated by $Al^{3+}$ at lower concentration and inhibited at

higher concentrations. Siegel and Haug (1983) and Siegel ***et al.*** (1983) reported that $Al^{3+}$ binds stoichiometrically and cooperatively to calmodulin, the normal activator of the enzyme and thereby induces a major structural change. Ganrot (1986) stated that inhibition of phosphodiesterase (ALP) is probably the result of a high affinity interaction between $Al^{3+}$ and calmodulin. Inhibition of alkaline phosphatase activity in ***Cyprinus carpio*** var. ***communis*** during sublethal exposure with aluminium may have been due to direct inhibition of enzymes which may find support from the observations of above workers.

# SUMMARY

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1. The 24 h median lethal concentration of aluminium sulphate to fish was 44.90 ppm and the same for 96 h was 26.42 ppm, indicating that the metal is toxic to fish.

2. Addition of median lethal concentration of aluminium sulphate reduced the pH of the test water from 7.5±1 to 4.9±1. In this pH range polymerisation of monomeric aluminium hydroxide species to higher molecular species may occur which ~~is~~ are highly toxic to fish.

3. Deposition of toxic aluminium polymers on the gill surface might have caused structural alterations such as edema, lamellar fusion of gills and profuse secretion of mucus causing severe clogging of the interlamellar spaces. The above changes might have resulted in impaired gaseous exchange in gills leading to mortality of fish during acute treatment.

4. $Na^+$ and $Cl^-$ ions were reduced in the plasma of fish exposed to acute and sublethal concentrations of aluminium. This may ~~be~~ have been due to the deposition of aluminium polymers on the gills leading to reduction in ionic exchange for transport ~~,or~~ due to the inhibition of active ion influx ~~,or~~ stimulation of passive efflux through paracellular channels or due to the inhibition of transporting enzyme in gills.

5 Elevation in plasma potassium in the present study may be due to rupture of cell membranes causing release of cellular potassium in to the plasma or reduction in the extracellular space or decrease in the plasma volume.

6. Blood pH and $HCO_3^-$ levels of fish treated with acute aluminium toxicity decreased significantly. However, in sublethal treatment the above changes were not significant. Surplus $H^+$ ion produced during aluminium hydrolysis, or aluminium induced hypoxia due to impaired gaseous exchange provoking lactic acid, or $PCO_2$ accumulation, or inhibition of acid-base relevant ion exchange might be the probable causes of blood pH decline observed in the present study. Decline of $HCO_3^-$ level in the plasma in the present study may be due to inhibition of carbonic anhydrous enzyme.

7. Fish exposed to both acute and sublethal studies exhibited reduced levels of hemoglobin, hematocrit and RBC count than that of the control. This may be due to disturbance in the fluid volume balance or inhibition of erythropoiesis, or erythroclasia or interference of hemometabolism by aluminium ions. Leucocytosis observed in the acute and sublethal treatments is probably due to stimulated immunological mechanism against aluminium stress. Swelling of red blood cells may be the reason for the significant changes in MCHC and MCV values in sublethal study.

8. The decrease in the plasma protein content in the present study during acute and sublethal treatments may be due to rapid utilisation of protein, or increased proteolysis, or reduction in the protein synthesis by the fish under aluminium

stress. During sublethal treatment hypoxia may be responsible for the elevation of plasma protein content which may act as an osmoeffector particle consequent to ion disturbance caused by aluminium toxicity.

9. Plasma glucose was elevated in both acute and sublethal treatments. This may been due to enhanced plasma cortisol level in the experimental fish resulting in glycogenolysis or gluconeogenesis to provide additional energy during the times of metabolic stress.

10. Gill and plasma $Na^+$ $K^+$ ATPase activity of fish was inhibited by aluminium toxicity both in acute and sublethal studies. This may be due to direct inhibition of enzyme by aluminium ions ~~or~~ replacement of normal co-factor $Mg^{2+}$ ions of the enzyme, ~~or~~ binding of the metal ion with the phosphate group of the enzyme or 'chloride cell' damage. The increase in the plasma $Na^+$ $K^+$ ATPase in the sublethal treatment after 21st day may be due to 'hormesis' (Yang and Randall, 1996) which may be a compensation process to inhibitory challenges produced by aluminium.

11. Both acid and alkaline phosphatases activities increased in the experimental fish which may be due to lysosomal membrane damage causing leakage of cellular enzyme into plasma. However, a decrease in the plasma ALP activity was recorded in the sublethal treatment. This may be attributed to direct enzyme inhibition by aluminium metal which is reported to have a high affinity interaction with calmodulin, the normal activator of the enzyme (Ganrot, 1986).

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